EXHIBIT 3-2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/00690

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FOWKE K R ET AL: "Genetic analysis of human DNA recovered from minute amounts of serum or plasma" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 180, no. 1, 13 March 1995, page 45-51 XP004021069 see abstract ---	1-3
P,X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US AN (NLM) 97420079, LO YM ET AL: "Presence of fetal DNA in maternal plasma and serum." XP002070361 cited in the application see abstract & LANCET, AUG 16 1997, 350 (9076) P485-7, ENGLAND, -----	1-3,6, 13,22-26

INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/GB 98/00690

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 08304 A (ISIS INNOVATION) 13 June 1991 cited in the application see abstract; claims	1-3,6,13
A	GB 2 299 166 A (ANKER PHILIPPE ;STROUN MAURICE (CH); VASIOUKHIN VALERI (US)) 25 September 1996 cited in the application see abstract; claims	1-3
A	WO 95 06137 A (AUSTRALIAN RED CROSS ;QUEENSLAND INST MED RES (AU); HYLAND CATHERI) 2 March 1995 see abstract; claims	1-3,10, 11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
3 July 1998	21/07/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. ational Application No
PCT/GB 98/00690

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9108304 A	13-06-1991	EP 0502037 A	09-09-1992
GB 2299166 A	25-09-1996	CH 686982 A	15-08-1996
		AU 1075695 A	03-07-1995
		WO 9516792 A	22-06-1995
WO 9506137 A	02-03-1995	AU 7486694 A	21-03-1995

PCT/GB98/00690

PA[illegible]T COOPERATION TREAT[illegible]

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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Crystal Plaza 2
Washington, DC 20231
États-Unis d'Amérique

in its capacity as elected Office

Date of mailing (day/month/year) 07 October 1998 (07.10.98)	
International application No. PCT/GB98/00690	**Applicant's or agent's file reference** KP/VM/2216 PCT
International filing date (day/month/year) 04 March 1998 (04.03.98)	**Priority date** (day/month/year) 04 March 1997 (04.03.97)
Applicant LO, Yuk-Ming, Dennis et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

17 September 1998 (17.09.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Yolaine CUSSAC
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992) 2269344

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
KP/VM/2216 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00690	04/03/1998	04/03/1997
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
ISIS INNOVATION LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   VI ☒ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/09/1998	16.12.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Goetz, M Telephone No. (+49-89) 2399-8697

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00690

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-38 as originally filed

Claims, No.:

1-26 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00690

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB98/00690

Re Item I

Basis of the report

The examination is being carried out on the **following application documents**:

Description, pages:
1-38 as originally filed

Claims, No.:
1-26 as originally filed

Drawings, sheets:
1/4-4/4 as originally filed

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

None of the documents cited in the International Search Report either discloses or suggests the key element of the present invention, i.e. the detection of foetal nucleic acid in the serum or plasma fraction of a maternal blood sample.

While it is true that the prior art already considered the possibility of diagnosing cancer by detecting tumour specific DNA mutations in the blood plasma fraction, there is no scientifically sound reason to believe that a skilled person would have automatically carried over this prior knowledge to the situation of prenatal diagnostic markers.

The subject-matter of present claims 1 - 26, based on the said key element, therefore complies with the requirements pursuant to Art. 33(2) and (3) PCT.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB98/00690

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Document	Publication date *(day/month/year)*
LO YM et al., "Presence of fetal DNA in maternal plasma and serum." LANCET 350(9076), p. 485-7	16/08/97

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
KP/VM/2216 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/00690	04/03/1998	04/03/1997
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
ISIS INNOVATION LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

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   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/09/1998	16.12.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Goetz, M Telephone No. (+49-89) 2399-8697

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00690

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

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Claims, No.:

1-26 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/00690

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB98/00690

Re Item I

Basis of the report

The examination is being carried out on the **following application documents**:

Description, pages:
1-38 as originally filed

Claims, No.:
1-26 as originally filed

Drawings, sheets:
1/4-4/4 as originally filed

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

None of the documents cited in the International Search Report either discloses or suggests the key element of the present invention, i.e. the detection of foetal nucleic acid in the serum or plasma fraction of a maternal blood sample.

While it is true that the prior art already considered the possibility of diagnosing cancer by detecting tumour specific DNA mutations in the blood plasma fraction, there is no scientifically sound reason to believe that a skilled person would have automatically carried over this prior knowledge to the situation of prenatal diagnostic markers.

The subject-matter of present claims 1 - 26, based on the said key element, therefore complies with the requirements pursuant to Art. 33(2) and (3) PCT.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/GB98/00690

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Document	Publication date *(day/month/year)*
LO YM et al., "Presence of fetal DNA in maternal plasma and serum." LANCET 350(9076), p. 485-7	16/08/97

Feb 11/09

PCT/GB98/00690

09/380696

The Patent Office

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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

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Signed A W Brewer

Dated 27 MAR 1998

Patents Form 1/77

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16)

The Patent Office

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

THE PATENT OFFICE D -4 MAR 1997 RECEIVED BY HAND

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference KP/VM/2216

-4 MAR 1997

2. Patent application number *(The Patent Office will fill in this part)* **9704444.0**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

ISIS INNOVATION LIMITED
2 South Parks Road
OXFORD
OX1 3UB
United Kingdom

Patents ADP number *(if you know it)* 5919564001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

NON-INVASIVE PRENATAL DIAGNOSIS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Stevens, Hewlett & Perkins
1 Serjeants' Inn
Fleet Street
London
EC4Y 1LL

Patents ADP number *(if you know it)* 1545003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	10
Claim(s)	1
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

STEVENS, HEWLETT & PERKINS

Signature Stevens, Hewlett & Perkins

Date 04.03.97

12. Name and daytime telephone number of person to contact in the United Kingdom 0171 936 2499 Kate Privett

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

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d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

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NON-INVASIVE PRENATAL DIAGNOSIS

This invention relates to prenatal diagnosis using non-invasive techniques. In particular, it relates to prenatal diagnosis by detecting foetal nucleic acids in serum or plasma from a maternal blood sample.

Conventional prenatal screening methods for detecting foetal abnormalities and for sex determination traditionally use foetal samples derived by invasive techniques such as amniocentesis and chorionic villus sampling. These techniques require careful handling and present a degree of risk to the mother and to the pregnancy.

More recently, techniques have been devised for predicting abnormalities in the foetus and possible complications in pregnancy, which use maternal blood or serum samples. Three markers commonly used include alpha-foetoprotein (AFP - of foetal origin), human chorionic gonadotrophin (hCG) and estriol, for screening for Down's Syndrome and neural tube defects. Maternal serum is also currently used for biochemical screening for chromosomal aneuploidies and neural tube defects. The passage of nucleated cells between the mother and foetus is now a well-recognised phenomenon (Lo *et al* 1989; Lo *et al* 1996). The use of foetal cells in maternal blood for non-invasive prenatal diagnosis (Simpson and Elias 1993) avoids the risks associated with conventional invasive techniques. WO 91/08304 describes prenatal genetic determination using foetal DNA obtained from foetal cells in the maternal blood. Considerable advances have been made in the enrichment and isolation of foetal cells for analysis (Simpson and Elias 1993; Cheung *et al* 1996). However, these techniques are time-consuming or require expensive equipment.

Recently, there has been interest in the use of plasma or serum-derived DNA for molecular diagnosis (Mulcahy *et al* 1996). In particular, it has been demonstrated that tumour DNA can be detected by

the polymerase chain reaction (PCR) in the plasma or serum of some patients (Chen *et al* 1996; Nawroz *et al* 1996).

GB 2 299 166 describes non-invasive cancer diagnosis by detection of K-*ras* and N-*ras* gene mutations using PCR-based techniques.

It has now been discovered that foetal DNA is detectable in maternal serum or plasma samples. This is a surprising and unexpected finding; maternal plasma is the very material that is routinely discarded by investigators studying non-invasive prenatal diagnosis using foetal cells in maternal blood. The detection rate is much higher using serum or plasma than using nucleated blood cell DNA extracted from a comparable volume of whole blood, suggesting that there is enrichment of foetal DNA in maternal plasma and serum. It is important that foetal DNA is found in maternal plasma as well as serum because this indicates that the DNA is not an artefact of the clotting process.

This invention provides a method of performing a prenatal diagnosis on a maternal serum or plasma sample, which method comprises detecting the presence of a nucleic acid sequence of foetal origin in the sample.

The term "prenatal diagnosis" as used herein covers determination of any maternal or foetal condition or characteristic which is related to either the foetal DNA itself or to the quantity or quality of the foetal DNA in the maternal serum or plasma. Included are sex determination, and detection of foetal abnormalities which may be for example chromosomal aneuploidies or simple mutations. Also included is detection and monitoring of pregnancy-associated conditions such as pre-eclampsia which may result in differing amounts of foetal DNA being present in the maternal serum or plasma. The nucleic acid detected in the method according to the invention may be of a type other than DNA e.g. mRNA.

The maternal serum or plasma sample is derived from the maternal blood. As little as 10μl of serum or plasma can be used. However it may be preferable to employ larger samples in order to increase accuracy. The volume of the sample required may be dependent upon the condition or characteristic being detected. In any case, the volume of maternal blood which needs to be taken is small.

The preparation of serum or plasma from the maternal blood sample is carried out by standard techniques. The serum or plasma is normally then subjected to a nucleic acid extraction process. Suitable methods include the boiling method described herein in the examples, and variations of that method. Possible alternatives include the controlled heating method described by Frickhofen and Young (1991). Two other suitable serum and plasma extraction methods include (i) proteinase K treatment followed by phenol/chloroform extraction; and (ii) extraction using a Qiamp Blood Kit. It is envisaged that serum and plasma nucleic acid extraction methods allowing the purification of DNA or RNA from a larger volume of maternal sample than described herein in the example, will increase the amount of foetal nucleic acid material for analysis and will thus improve the accuracy. A sequence-based enrichment method could also be used on the maternal serum or plasma to specifically enrich for foetal nucleic acid sequences.

An amplification of foetal DNA sequences in the sample is normally carried out. Standard nucleic acid amplification systems can be used, including PCR, the ligase chain reaction, nucleic acid sequence based amplification (NASBA), branched DNA methods, and so on. Preferred amplification methods involve PCR.

The method according to the invention may be particularly useful for sex determination which may be carried out by detecting the presence of a Y chromosome. It is demonstrated herein that using only 10μl of plasma or serum a detection rate of 80% for plasma and 70% for

serum can be achieved. The use of just 1ml of maternal plasma or serum will result in a 100-fold increase in the absolute amount of foetal genetic material available for analysis. This is expected to provide a very accurate system for detecting paternally-inherited foetal DNA sequences.

The method according to the invention can be applied to the detection of any paternally-inherited sequences which are not possessed by the mother. Examples include:

a) Foetal rhesus D status determination in rhesus negative mothers (Lo *et al* 1993). This is possible because rhesus D positive individuals possess the rhesus D gene which is absent in rhesus D negative individuals. Therefore, the detection of rhesus D gene sequences in the plasma and serum of a rhesus D negative mother is indicative of the presence of a rhesus D positive foetus. This approach may also be applied to the detection of foetal rhesus D mRNA in maternal plasma and serum.

b) Haemoglobinopathies (Camaschella *et al* 1990). Over 450 different mutations in the beta-globin gene have been known to cause beta-thalassaemia. Provided that the father and mother carry different mutations, the paternal mutation can be used as an amplification target on maternal plasma and serum, so as to assess the risk that the foetus may be affected.

c) Paternally-inherited DNA polymorphisms or mutations. Paternally-inherited DNA polymorphisms or mutations present on either a Y or a non-Y chromosome, can be detected in maternal plasma and serum to assess the risk of the foetus being affected by a particular disease by linkage analysis. Furthermore, this type of analysis can also be used to ascertain the presence of foetal nucleic acid in a particular maternal plasma or serum sample, prior to diagnostic analysis such as sex determination. This application will require the prior genotyping of the father and mother using a panel of

polymorphic markers and then an allele for detection will be chosen which is present in the father, but is absent in the mother.

The plasma or serum-based non-invasive prenatal diagnosis method according to the invention can be applied to the screening of Down's Syndrome and other chromosomal aneuploidies. Two possible ways in which this might be done are as follows:

a) It has been found that in pregnancy involving foetuses with chromosomal aneuploidies e.g. Down's Syndrome, the level of foetal cells circulating in maternal blood is higher than in pregnancies involving normal foetuses (Bianchi *et al* 1996). Following the surprising discovery disclosed herein that foetal DNA is present in maternal plasma and serum, it may be expected that the level of foetal DNA in maternal plasma and serum will be higher in pregnancies where the foetus has a chromosomal aneuploidy than in normal pregnancies. Quantitative detection of foetal nucleic acid in the maternal plasma or serum e.g. a quantitative PCR assay, could be used to screen pregnant women for chromosomal aneuploidies.

b) A second method involves the quantitation of foetal DNA markers on different chromosomes. For example, for a foetus affected by Down's Syndrome the absolute quantity of foetal chromosomal 21-derived DNA will always be greater than that from the other chromosomes. The recent development of very accurate quantitative PCR techniques, such as real time quantitative PCR (Heid *et al* 1996) will allow the realisation of this type of analysis.

Another potential application of the accurate quantitation of foetal nucleic acid levels in the maternal serum or plasma is in the molecular monitoring of certain placental pathologies, such as pre-

eclampsia. It is likely that placental damage in pre-eclampsia may result in alterations in foetal DNA concentration in material serum and plasma.

It is anticipated that it will be possible to incorporate the nucleic acid-based diagnosis methods described herein into existing prenatal screening programmes. Sex determination has successfully been performed on pregnancies from 12 to 40 weeks of gestation.

The invention will now be illustrated in the following Example, which does not in any way limit the scope of the invention.

EXAMPLE

METHODS

Patients

Pregnant women attending the Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford were recruited prior to amniocentesis or delivery. Ethics approval of the project was obtained from the Central Oxfordshire Research Ethics Committee. Informed consent was sought in each case. Five to ten ml of maternal peripheral blood was collected into an EDTA and a plain tube. For women undergoing amniocentesis, maternal blood was always collected prior to the procedure and 10 ml of amniotic fluid was also collected for foetal sex determination. For women recruited just prior to delivery, foetal sex was noted at the time of delivery. Control blood samples were also obtained from 10 non-pregnant female subjects and further sample processing was as for specimens obtained from pregnant individuals.

Sample preparation

Maternal blood samples were processed between 1 to 3 hours following venesection. Blood samples were centrifuged at 3000g and plasma and serum were carefully removed from the EDTA-containing and plain tubes, respectively, and transferred into plain polypropylene

tubes. Great care was taken to ensure that the buffy coat or the blood clot was undisturbed when plasma or serum samples, respectively, were removed. Following removal of the plasma samples, the red cell pellet and buffy coat were saved for DNA extraction using a Nucleon DNA extraction kit (Scotlabs, Strathclyde, Scotland, U.K.). The plasma and serum samples were then subjected to a second centrifugation at 3000g and the recentrifuged plasma and serum samples were collected into fresh polypropylene tubes. The samples were stored at -20^{o}C until further processing.

DNA extraction from plasma and serum samples

Plasma and serum samples were processed for PCR using a modification of the method of Emanuel and Pestka (1993). In brief, 200 µl of plasma or serum was put into a 0.5ml eppendorf tube. The sample was then heated at 99^{o}C for 5 minutes on a heat block. The heated sample was then centrifuged at maximum speed using a microcentrifuge. The clear supernatant was then collected and 10 µl was used for PCR.

DNA extraction from amniotic fluid

The amniotic fluid samples were processed for PCR using the method of Rebello *et al* (1991). One hundred µl of amniotic fluid was transferred into a 0.5 ml eppendorf tube and mixed with an equal volume of 10% Chelex-100 (Bio-Rad). Following the addition of 20 µl of mineral oil to prevent evaporation, the tube was incubated at 56°C for 30 minutes on a heat block. Then, the tube was vortexed briefly and incubated at 99°C for 20 minutes. The treated amniotic fluid was stored at 4°C until PCR and 10 µl was used in a 100µl reaction.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was carried out essentially as described (Saiki *et al* 1988) using reagents obtained from a

GeneAmp DNA Amplification Kit (Perkin Elmer, Foster City, CA, USA). The detection of Y-specific foetal sequence from maternal plasma, serum and cellular DNA was carried out as described using primers Y1.7 and Y1.8, designed to amplify a single copy Y sequence (DYS14) (Lo *et al* 1990). The sequence of Y1.7 is 5' CAT CCA GAG CGT CCC TGG CTT 3' and that of Y1.8 is 5' CTT TCC ACA GCC ACA TTT GTC 3'. The Y-specific product was 198 bp. Sixty cycles of Hot Start PCR using Ampliwax technology were used on 10 μl of maternal plasma or serum or 100 ng of maternal nucleated blood cell DNA (denaturation step of 94°C 1 minute and a combined reannealing/extension step of 57°C 1 minute). Forty cycles were used for amplification of amniotic fluid. PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining. PCR results were scored before the foetal sex was revealed to the investigator.

Results

Sensitivity of PCR assay

Serial dilutions of male genomic DNA in 1 μg of female genomic DNA were performed and amplified by the Y-PCR system using 60 cycles of amplification. Positive signals were detected up to the 100,000 dilution, i.e., approximately the equivalent of a single male cell.

Amplification of foetal DNA sequence from maternal plasma and serum

Maternal plasma and serum samples were collected from 43 pregnant women with gestational ages from 12 to 40 weeks. There were 30 male foetuses and 13 female foetuses. Of the 30 women bearing male foetuses, Y-positive signals were detected in 24 plasma samples and 21 serum samples, when 10 μl of the respective samples was used for PCR. When nucleated blood cell DNA was used for Y-PCR, positive signals were only detected in 5 of the 30 cases. None of the 13 women bearing female foetuses and none of the 10 non-pregnant female controls resulted in a

positive Y signal when either plasma, serum or cellular DNA was amplified. Accuracy of this technique, even with serum/plasma samples of only 10 µl, is thus very high and most importantly it is high enough to be useful. It will be evident that accuracy can be improved to 100% or close to 100%, for example by using a larger volume of serum or plasma.

References

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Camaschella C, Alfarano A, Gottardi E, *et al.* Prenatal diagnosis of fetal hemoglobin Lepore-Boston disease on maternal peripheral blood. Blood 1990; **75**: 2102-106.

Chen X Q, Stroun M, Magnenat J-L, *et al.* Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996; **2**: 1033-35.

Cheung M C, Goldberg JD, Kan YW. Prenatal diagnosis of sickle cell anemia and thalassemia by analysis of fetal cells in maternal blood. Nat Genet 1996; **14**: 264-68.

Emanuel S L, Pestka S. Amplification of specific gene products from human serum. GATA 1993; **10**: 144-46.

Frickhofen N. & Young N.S. A rapid method of sample preparation for detection of DNA viruses in human serum by polymerase chain reaction. J.Virological Methods 1991; **35**: 65-72.

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Lo Y M D, Lo E S F, Watson N, *et al.* Two-way cell traffic between mother and fetus: biologic and clinical implications. Blood 1996; **88**: 4390-95.

Lo Y M D, Patel P, Sampietro M, Gillmer M D G, Fleming K A, Wainscoat JS. Detection of single-copy fetal DNA sequence from maternal blood. Lancet 1990; **335**: 1463-64.

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Mulcahy H E, Croke DT, Farthing M J G, Cancer and mutant DNA in blood plasma. Lancet 1996; **348**: 628.

Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996; **2**: 1035-37.

Rebello M T, Hackett G, Smith J, *et al.* Extraction of DNA from amniotic fluid cells for the early prenatal diagnosis of genetic disease. Prenat Diagn 1991; **11**: 41-46.

Saiki R K, Gelfand D H, Stoffel S, *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988; **239**: 487-91.

Simpson J L, Elias S. Isolating fetal cells from maternal blood: advances in prenatal diagnosis through molecular technology. JAMA 1993; **270**: 2357-61.

CLAIMS:

1. A method of performing a prenatal diagnosis on a maternal serum or plasma sample, which method comprises detecting the presence of a nucleic acid sequence of foetal origin in the sample.

2. The method according to claim 1, wherein the foetal nucleic acid sequence is amplified prior to detection.

3. The method according to claim 2, wherein the foetal nucleic acid sequence is amplified by the polymerase chain reaction.

4. The method according to claim 2 or claim 3, wherein at least one foetal sequence specific oligonucleotide primer is used.

5. The method according to any one of claims 1 to 4, wherein the foetal nucleic acid sequence is from the Y chromosome.

6. The method according to any one of claims 1 to 4, wherein the foetal nucleic acid is from a paternally-inherited non-Y chromosome.

7. The method according to any one of claims 1 to 5, for the purpose of sex determination of the foetus.

8. The method according to any one of claims 1 to 6, for detecting a genetic abnormality in the foetus.

9. The method according to any one of claims 1 to 8, wherein the foetal nucleic acid sequence is DNA.

10. The method according to any one of claims 1 to 9, wherein a nucleic acid extraction step is performed on the serum or plasma sample.

11. The method according to claim 10, wherein the nucleic acid extraction step includes heating the serum or plasma sample.

P. .NT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
KP/VM/2216 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 98/ 00690	04/03/1998	04/03/1997
Applicant		
ISIS INNOVATION LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

Interr al Application No
PCT/ 98/00690

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 08304 A (ISIS INNOVATION) 13 June 1991 cited in the application see abstract; claims	1-3,6,13
A	GB 2 299 166 A (ANKER PHILIPPE ;STROUN MAURICE (CH); VASIOUKHIN VALERI (US)) 25 September 1996 cited in the application see abstract; claims	1-3
A	WO 95 06137 A (AUSTRALIAN RED CROSS ;QUEENSLAND INST MED RES (AU); HYLAND CATHERI) 2 March 1995 see abstract; claims	1-3,10, 11

-/--

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 July 1998	21/07/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr ...l Application No
PCT/... 98/00690

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	FOWKE K R ET AL: "Genetic analysis of human DNA recovered from minute amounts of serum or plasma", JOURNAL OF IMMUNOLOGICAL METHODS, vol. 180, no. 1, 13 March 1995, page 45-51 XP004021069 see abstract ---	1-3
P,X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US AN (NLM) 97420079, LO YM ET AL: "Presence of fetal DNA in maternal plasma and serum." XP002070361 cited in the application see abstract & LANCET, AUG 16 1997, 350 (9076) P485-7, ENGLAND, -----	1-3,6, 13,22-26

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Informa i patent family members

Intern l Application No
PCT/GB 98/00690

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9108304 A	13-06-1991	EP 0502037 A	09-09-1992
GB 2299166 A	25-09-1996	CH 686982 A	15-08-1996
		AU 1075695 A	03-07-1995
		WO 9516792 A	22-06-1995
WO 9506137 A	02-03-1995	AU 7486694 A	21-03-1995

Express Mail Label No.: EL375088070US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	
Lo et al.	Our File: SHP-PT048
Application No.: Not Yet Known	Date: September 2, 1999
Filed: Not Yet Known	
For: NON-INVASIVE PRENATAL DIAGNOSIS	
Group: Not Yet Known	
Examiner: Not Yet Known	

COMMUNICATION UNDER RULE 37 C.F.R. § 1.53(b)

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The purpose of this Communication is to advise the Office that the above-identified application is being filed pursuant to 37 C.F.R. § 1.53(b) with an unsigned Declaration and Power of Attorney. It is respectfully requested that the application be granted a filing date of even date with this Communication.

Respectfully submitted,

Lo et al.

By _____
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/tc
Enclosures

Express Mail Label No. EL375088070US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:		
	Lo et al.	Our File: SHP-PT048
Application No.:	Not Yet Known	Date: September 3, 1999
Filed:	Not Yet Known	
For:	NON-INVASIVE PRENATAL DIAGNOSIS	
Group:	Not Yet Known	
Examiner:	Not Yet Known	

CERTIFICATE OF MAILING
BY EXPRESS MAIL ACCOMPANYING PATENT APPLICATION

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby certify that the accompanying correspondence is being deposited with the "Express Mail Post Office to Addressee" service of the United States Postal Service in an envelope addressed to Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231 on September 3, 1999. The number of the "Express Mail" mailing label EL375088070US has been placed on the accompanying correspondence prior to mailing. It is therefore respectfully requested that this correspondence be considered as having been filed in the Office on the date shown above in accordance with the provisions of 37 C.F.R. § 1.10.

Respectfully submitted,

9/3/99
Date

Anthony Pentasuglio

VOLPE and KOENIG, P.C.
400 One Penn Center
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410 Rec'd PCT/PTO 03 SEP 1999

Express Mail Label No.: EL375088070US

FORM PTO-1390 (REV 11-98) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: SHP-PT048

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/380696 Not Yet Known

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB98/00690	4 March 1998	4 March 1997

TITLE OF INVENTION: NON-INVASIVE PRENATAL DIAGNOSIS

APPLICANT(S) FOR DO/EO/US: Lo et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An unsigned oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    Communication Under Rule 37 C.F.R. Section 1.53(b);
    International Search Report (included with International Publication);
    International Preliminary Examination Report; and
    Certificate of Mailing by Express Mail.

09380696 090899

514 Rec'd PCT/PTO 03 SEP 1999

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/380696 Not Yet Known	INTERNATIONAL APPLICATION NO. PCT/GB98/00690	ATTORNEY'S DOCKET NUMBER SHP-PT048

17. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$760.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	26 - 20 =	6	X $18.00	$ 108.00	
Independent claims	3 - 3 =	0	X $78.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 948.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 948.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 948.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 948.00	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 22-0493 in the amount of $948.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 22-0493. A duplicate copy of this sheet is enclosed.

Our order no. is 683

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

SIGNATURE

C. Frederick Koenig III, Esquire
NAME

29,662
REGISTRATION NUMBER

09/380696

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: ASSISTANT COMMISSIONER FOR PATENTS
Box PCT
Washington, D.C. 20231

U.S. APPLICATION NO		FIRST NAMED APPLICANT	ATTY. DOCKET NO
09/380,696	LO	Y	SHP-PT048

5071

VOLPE AND KOENIG
400 ONE PENN CENTER
1617 JOHN F KENNEDY BOULEVARD
PHILADELPHIA PA 19103

INTERNATIONAL APPLICATION NO	
PCT/GB98/00590	
INTERNATIONAL FILING DATE	PRIORITY DATE
03/02/98	03/04/97

10/28/99
DATE MAILED.

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

1. The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as ☐ a Designated Office (37 CFR 1.494), ☒ an Elected Office (37 CFR 1.495):

- ☒ U.S. Basic National Fee.
- ☒ Copy of the international application in:
  - ☐ a non-English language.
  - ☒ English.
- ☐ Translation of the international application into English.
- ☐ Oath or Declaration of inventors(s) for DO/EO/US.
- ☐ Copy of Article 19 amendments.
- ☐ Translation of Article 19 amendments into English.
- ☒ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Translation of Annexes to the International Preliminary Examination Report into English.
- ☒ Preliminary amendment(s) filed 03 SEP 1999 and _____.
- ☒ Information Disclosure Statement(s) filed 03 SEP 1999 and _____.
- ☐ Assignment document.
- ☐ Power of Attorney and/or Change of Address.
- ☐ Substitute specification filed _____.
- ☐ Statement Claiming Small Entity Status.
- ☒ Priority Document.
- ☒ Copy of the International Search Report ☒ and copies of the references cited therein.
- ☐ Other:

2. The following items **MUST** be furnished within the period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- ☐ a. Translation of the application into English. Note a processing fee will be required if submitted later than the appropriate 20 or 30 months from the priority date.
  - ☐ The current translation is defective for the reasons indicated on the attached Notice of Defective Translation.
- ☐ b. Processing fee for providing the translation of the application and/or the Annexes later that the appropriate 20 or 30 months from the priority date (37 CFR 1.492(f)).
- ☒ c. Oath or declaration of the inventors, in compliance with 37 CFR 1.497(a) and (b), identifying the application by the International application number and international filing date.
  - ☐ The current oath or declaration does not comply with 37 CFR 1.497(a) and (b) for the reasons indicated on the attached PCT/DO/EO/917.
- ☒ d. Surcharge for providing the oath or declaration later that the appropriate 20 or 30 months from the priority date (37 CFR 1.492(e)).

3. Additional claim fees of $_____ as a ☐ large entity ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due (37 CFR 1.492(g)). See attached PTO-875.

ALL OF THE ITEMS SET FORTH IN 2(a)-2(d) AND 3 ABOVE MUST BE SUBMITTED WITHIN ONE MONTH FROM THE DATE OF THIS NOTICE OR BY ☐ 21 OR ☒ 31 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

4. Translation of the Annexes MUST be submitted no later that the time period set above or the annexes will be cancelled. Note processing fee will be required if submitted later than 30 months from the priority date.

5. ☐ The Article 19 amendments are cancelled since a translation was not provided by the appropriate 20 (37 CFR 1.494(d)) or 30 (37 CFR 1.495(d)) months from the priority date.

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above. (37 CFR 1.5)

A copy of this notice MUST be returned with this response.

Enclosed: ☐ PCT/DO/EO/917 ☐ Notice of Defective Translation
☐ PTO-875

Linda Wilbur
PCT International Division
Telephone: (703)

FORM PCT/DO/EO/905 (December 1997)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:		
	Lo et al.	Our File: SHP-PT048
Application No.:	09/380,696	Date: November 23, 1999
Filed:	Not Yet Known	
For: NON-INVASIVE PRENATAL DIAGNOSIS		
Group:	Not Yet Known	
Examiner:	Not Yet Known	

COMMUNICATION IN RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US) dated October 28, 1999, enclosed herewith are the following:

1. Copy of the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US);
2. A fully executed Declaration and Power of Attorney for Patent Application; and
3. A check in the amount of $65 as payment of the surcharge for a small entity.

Applicant: Lo et al.
Application No.: 09/380,696

Small entity verification has been submitted in conjunction with a Request for Refund filed November 2, 1999.

In the event that any additional fees are required with respect to this Communication, or in the event of an overpayment, please charge such additional fees or credit such overpayments to the Deposit Account of the undersigned, No. 22-0493, under our Order No. 1353. Two copies of this Communication are enclosed.

In accordance with the above, applicant awaits receipt of the Notification of Acceptance in this matter.

Respectfully submitted,

Lo et al.

By _____
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/ras
Enclosures (5)

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	SHP-PT048
First Named Inventor	Lo et al.
COMPLETE IF KNOWN	
Application Number	09/380,696
Int'l. Filing Date	March 4, 1998
Group Art Unit	Not Yet Known
Examiner Name	Not Yet Known

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NON-INVASIVE PRENATAL DIAGNOSIS

(Title of the invention)

the specification of which
☐ is attached hereto
OR
☒ was filed on (MM/DD/YYYY) 03/04/1998 as United States Application Number or PCT International Application Number PCT/GB98/00690 and was amended on (MM/DD/YYYY) 09/03/99 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
9704444.0	Great Britian	03/04/1997	☐	☐	☒
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Please type a plus sign (+) inside this box → [+]

PTO/SB/01 (12-97)
Approved for use through 9/30/00. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
PCT/GB98/00690	03/04/1998	

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number → *Place Customer Number Bar Code Label here*
OR
☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Alfred Stapler	16,675	Glenn M. Massina	40,081
Anthony S. Volpe	26,377	Marilou E. Watson	~~42,913~~
C. Frederick Koenig III	29,662	Jeffrey M. Glabicki	42,584
Gerald B. Halt, Jr.	37,633		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label OR ☒ Correspondence address below

Name	C. Frederick Koenig III, Esquire VOLPE and KOENIG, P.C.				
Address	400 One Penn Center				
Address	1617 John F. Kennedy Blvd.				
City	Philadelphia	State	PA	ZIP	19103
Country	U.S.A.	Telephone	(215) 568-6400	Fax	(215) 568-6499

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Yuk-Ming Dennis	Lo

Inventor's Signature	[signature]					Date	23 Nov 1999
Residence: City	Homantin, Kowloon	State	Hong Kong	Country	China	Citizenship	British
Post Office Address	Department of Chemical Pathology						
Post Office Address	The Chinese University of Hong Kong, Prince of Wales Hospital						
City	Shatin, New Ter.	State	Hong Kong	ZIP		Country	China

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

09/381199

Please type a plus sign (+) inside this box → [+]

PTO/SB/02A (3-97)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
James Stephen		Wainscoat					
Inventor's Signature	J Wainscoat.					Date	23/11/99
Residence: City	Oxford GBX	State		Country	United Kingdom	Citizenship	British
Post Office Address	14 Woodlands Close						
Post Office Address							
City	Oxford	State		ZIP	OX3 7RY	Country	United Kingdom

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		ZIP		Country	

Burden Hour Statement: This form is estimated to take 0.4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

File History Report

☐ Paper number _____ is missing from the United States Patent and Trademark Office's original copy of the file history. No additional information is available.

☒ The following page(s) **Copy of Notification of missing Requirements** of paper number _____ is/are missing from the United States Patent and Trademark Office's original copy of the file history. No additional information is available

Additional comments: _____

520 Rec'd PCT/PTO 29 NOV 1999

Please type a plus sign (+) inside this box → [+]

PTO/SB/21 (6-98)
Approved for use through 09/30/2000. OMB 0651-0031
Patent and Trademark Office. U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/380,696
Filing Date	Not Yet Known
First Named Inventor	Lo et al.
Group Art Unit	Not Yet Known
Examiner Name	Not Yet Known
Total Number of Pages in This Submission: 8	Attorney Docket Number: SHP-PT048

ENCLOSURES *(check all that apply)*

- [x] Fee Transmittal Form
- [x] Fee Attached
- [ ] Amendment / Response
  - [ ] After Final
  - [ ] Affidavit(s)/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [x] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition Routing Slip (PTO/SB/69) and Accompanying Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Small Entity Statement
- [ ] Request for Refund
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Additional Enclosure(s) *(please identify below)*

Copy of Notification of Missing Requirements; and Fully Executed Declaration for Patent Application.

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	C. Frederick Koenig III, Esquire Reg. No. 29,662 VOLPE and KOENIG, P.C.
Signature	
Date	11/23/99

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231 on this date: November 23, 1999

Typed or printed name	C. Frederick Koenig III, Esquire		
Signature		Date	11/23/99

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be send to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

12/02/1999 PVOLPE 00000375 220493 09380696
01 FC:154 65.00 CH
65.00 DP

PTO/PCT Rec'd 29 NOV 1999

6/380696

#3

Volpe and Koenig, P.C. Revision of: PTO/SB/17 (12-98)
Approved for use through 09/30/2000. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 1999

Patent fees are subject to annual revision.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12.

TOTAL AMOUNT OF PAYMENT $65

Complete if Known	
Application Number	09/380,696
Filing Date	Not Yet Known
First Named Inventor	Lo et al.
Examiner Name	Not Yet Known
Group / Art Unit	Not Yet Known
Attorney Docket No.	SHP-PT048

METHOD OF PAYMENT (check one)

1. The Commissioner is hereby authorized to:
☐ charge the fees indicated hereon

Deposit Account Number: 22-0493
Deposit Account Name: VOLPE and KOENIG, P.C.

☒ Charge Any Deficiency or Credit Any Overpayment in the Total Fees Associated with this Communication
Our Order No. 1353

2. ☒ **Payment Enclosed:**
☒ Check ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	760	201	380	Utility filing fee	
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	760	208	380	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1)

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below	Fee Paid
Total Claims	-__	**=	X	=
Independent Claims	-__	**=	X	=
Multiple Dependent				=

***or number previously paid, if greater; For Reissues, see below*

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) -65.00 DP

Adjustment date: 12/02/1999 PVOLPE
12/02/1999 PVOLPE 00000001 09380696
01 FC:254

12/02/1999 PVOLPE 00000001 09380696
01 FC:254

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	$65
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	760	246	380	Filing a submission after final rejection (37 CFR 1 129(a))	
149	760	249	380	For each additional invention to be examined (37 CFR 1.129(b))	
Other fee (specify)					
Other fee (specify)					

* Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** $65

SUBMITTED BY				Complete (if applicable)	
Typed or Printed Name	C. Frederick Koenig III, Esquire			Reg. Number	29,662
Signature		Date	11/23/99	Deposit Account User ID	22-0493 VOLPE and KOENIG, P.C.

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

09/380696

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address. ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C 20231

U S APPLICATION NO 09/380696	LO	FIRST NAMED APPLICANT	ATTY DOCKET NO PT048

5071

VOLPE AND KOENIG
400 ONE PENN CENTER
1617 JOHN F KENNEDY BOULEVARD
PHILADELPHIA PA 19103

INTERNATIONAL APPLICATION NO	
PCT/GB98/00690	

U.S. FILING DATE	PRIORITY DATE
03/04/98	03/04/97

12/20/99
DATE MAILED

NOTIFICATION OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

1. The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as ☐ a Designated Office (37 CFR 1.494), ☒ an Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is **ACCEPTED** for national patentability examination in the United States Patent and Trademark Office.

2. The United States Application Number assigned to the application is shown above and the relevant dates are:

29 NOV 1999	29 NOV 1999
35 U.S.C. 102(e) DATE	DATE OF RECEIPT OF 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371(C) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

3. ☐ A request for immediate examination under 35 U.S.C. 371(f) was received on _____ and the application will be examined in turn.

4. The following items have been received:
- ☒ U.S. Basic National Fee.
- ☒ Copy of the international application in:
  - ☐ a non-English language.
  - ☒ English.
- ☐ Translation of the international application into English.
- ☒ Oath or Declaration of inventors(s) for DO/EO/US.
- ☐ Copy of Article 19 amendments. ☐ Translation of Article 19 amendments into English.
  The Article 19 amendments ☐ have ☐ have not been entered.
- ☒ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Copy of the Annexes to the International Preliminary Examination Report (IPER).
  - ☐ Translation of Annexes to the IPER into English.
  The Annexes ☐ have ☐ have not been entered.
- ☒ Preliminary amendment(s) filed 03 SEP 1999 and _____.
- ☒ Information Disclosure Statement(s) filed 03 SEP 1999 and _____.
- ☐ Assignment document.
- ☐ Power of Attorney and/or Change of Address.
- ☐ Substitute specification filed _____.
- ☐ Statement Claiming Small Entity Status.
- ☒ Priority Document
- ☒ Copy of the International Search Report ☒ and copies of the references cited therein.
- ☐ Other:

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

Vonda Wallace VW
PCT International Division
Telephone (703) 305-9[illegible]

FORM PCT/DO/EO/903 (December 1997)

DO/ EO WORKSHEET

U.S. Appl. No. 09/380696 International Appl. No. GB98/00690

Application filed by : ☐ 20 months ☒ 30 months

WIPO PUBLICATION INFORMATION

Publication No.: WO 98/39474 Publication Language : English Screening Done by : V.W

Publication Date : 11 Sept 98 Not Published : ☐ U.S. only designated ☐ EP request

INTERNATIONAL APPLICATION PAPERS IN THE APPLICATION FILE :

- ☒ International Application *(RECORD COPY)*
- ☒ Article 19 Amendments
- ☒ PCT/IB/331
- ☒ PCT/IPEA/409 IPER (PCT/IPEA/416 on front)
- ☐ Annexes to 409
- ☒ Priority Document (s) No. 1
- ☐ International Appl. on Double Sided Paper *(COPIES MADE)*
- ☐ Request form PCT/RO/101
- ☐ PCT/ISA/210 - Search Report
- ☐ Search Report References
- ☐ Other : _____

RECEIPTS FROM THE APPLICANT *(other than checked above) :*

- ☒ Baisc National Fee *(paid or authorized to charge)*
  - ☒ Description
  - ☒ Claims
  - ☐ Words in the Drawing Figure(s)
  - ☐ Article 19 Amendments
  - ☐ Annexes to 409 ☐ entered ☐ not entered
- ☐ Oath/ Declaration (executed)
- ☐ DNA Diskette
- ☒ Preliminary Amendment(s) Filed on : _____
- ☒ Information Disclosure Statement(s) Filed on : _____
- ☐ Assignment Document
- ☐ Power of Attorney/ Change of Address
- ☐ Substitute Specification Filed on : _____
- ☐ Verified Small Status Claim *(if submitted after Receipt Date - Is it timely ? Y/N)*
- ☐ Other : _____

NOTES : MISSED IA FROM IB Appl. Copy DOUBLE SIDED

35 U.S.C, 371 - Receipt of Request (PTO-1390)	03 SEP 1999
Date Acceptable Oath/ Declaration Received	29 NOV 1999
Date Complete 35 U.S.C. 371	29 NOV 1999
102(e) Date	29 NOV 1999
Date of Completion of DO/ EO 906 - Notification of Missing 102(e) Requirements	
Date of Completion of DO/ EO 907 - Notification of Acceptance for 102(e) Date	
Date of Completion of DO/ EO 911 - Application Accepted Under 35 U.S.C. 111	
Date of Completion of DO/ EO 905 - Notification of Missing Requirements	10/21/99
Date of Completion of DO/ EO 916 - Notification of Defective Response	
Date of Completion of DO/ EO 903 - Notification of Acceptance	12/15/99
Date of Completion of DO/ EO 909 - Notification of Abandonment	

DO/EO BIBLIOGRAPHIC DATA ENTRY

SERIAL NUMBER: 09 / 380696 RECEIPT DATE: 09 / 03 / 99
IA NUMBER: PCT/ GB98 / 00690 IA FILING DATE: 03 / 04 / 98
FAMILY NAME: LO DELAY WAIVED (Y/N): N
GIVEN NAME: YUK-MING DENNIS DEMAND RECEIVED (Y/N): Y
PRIORITY CLAIMED (Y/N): Y PRIORITY DATE: 03 / 04 / 97
NO BASIC FEE (Y/N): N US DESIGNATED ONLY (Y/N): N
ATTORNEY DOCKET NUMBER: SHP-PT048 COUNTRY: GBX
CORRESPONDENCE NAME/ADDRESS: CUSTOMER NUMBER: TELEPHONE
FAX
NAME: VOLPE AND KOENIG

STREET: 400 ONE PENN CENTER
1617 JOHN F KENNEDY BOULEVARD
CITY: PHILADELPHIA
STATE/COUNTRY: PA ZIP: 19103
MAIL:
APPLICATION TITLES:
NON-INVASIVE PRENATAL DIAGNOSIS

TAB TO LAST POSITION, PUSH SEND

Express Mail Label No.: EL375088070US
PATENT

#50
25.00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	Lo et al.	
Application No.:	Not Yet Known	Our File: SHP-PT048
Filed:	Not Yet Known	Date: September 3, 1999
For:	NON-INVASIVE PRENATAL DIAGNOSIS	
Group:	Not Yet Known	
Examiner:	Not Yet Known	

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination and before the calculation of the filing fee, please amend the application as follows:

IN THE CLAIMS

Please amend the claims as follows:

(Amended) The method according to claim 1 [or claim 3], wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification.

(Amended) The method according to [any one of claims 1 to 4] claim 1, wherein the foetal nucleic acid is detected by means of a sequence specific probe.

Applicants: Lo et al.
Application No.: Not Yet Known

Early consideration and allowance of this application are respectfully requested.

Respectfully submitted,

Lo et al.

By _____
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/tc
Enclosures

16 17. (Amended) The method according to [any one of claims 14 to 16] claim 13, wherein the pattern of variation of foetal DNA concentration in the maternal serum or plasma at particular stages of gestation is different from normal.

Q4 cont'd

17 18. (Amended) The method according to [claim 16 or claim 17] claim 13, for detection of pre-eclampsia.

18 19. (Amended) The method according to [claim 16 or claim 17] claim 13, for detection of a foetal chromosomal aneuploidy.

19 20. (Amended) The method according to [any one of claims 1 to 19] claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.

Q5

21 22. (Amended) A method of performing a prenatal diagnosis, which method comprises the steps of:

(i) providing a maternal blood sample;

(ii) separating the sample into a cellular and a non-cellular fraction;

(iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of [any one of claims 1 to 21] claim 1.

Applicant: Lo et al.
Application No.: Not Yet Known

a5 cont'd

(iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.

REMARKS

This Preliminary Amendment amends the claims as set forth in the PCT application, filed on March 4, 1998 to delete reference to multiple dependant claims.

Early consideration and allowance of claims 1-26 are respectfully requested.

Respectfully submitted,

Lo et al.

By______________________
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

09380696.112999

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/tc

47

A

PATENT #6 25.00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	
Lo et al.	Our File: SHP-PT048
Application No.: Not Yet Known	Date: September 2, 1999
Filed: Not Yet Known	
For: NON-INVASIVE PRENATAL DIAGNOSIS	
Group: Not Yet Known	
Examiner: Not Yet Known	

INFORMATION DISCLOSURE STATEMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Further to Applicants' duty of disclosure pursuant to 37 C.F.R. § 1.56, Applicants wish to bring to the Examiner's attention the material cited on the enclosed form PTO-1449. It is respectfully requested that the Examiner initial the form PTO-1449 upon consideration of the cited references and return an initialed copy with the next correspondence.

The references listed on the enclosed form PTO-1449 were cited in the International Search Report dated July 3, 1998. A copy of the Search Report showing the relevancy of each cited reference is also enclosed.

Express Mail Label No.: EL375088070US

Sheet 1 of 1

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use several sheets if necessary)	ATTY. DOCKET NO. SHP-PT048	SERIAL NO. 09/380696
	APPLICANT Lo et al.	
	FILING DATE Not Yet Known	GROUP Not Yet Known

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
JC	AA	9 1 0 8 3 0 4	06/91	PCT	C12Q	1/68		
JC	AB	9 5 0 6 1 3 7	03/95	PCT	C12Q	1/68		
JC	AC	2 2 9 9 1 6 6	09/96	GB	C12Q	1/68		

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

JC	AD		Journal of Immunological Methods; Vol. 180, No. 1; Fowke et al.; "Genetic Analysis Of Human DNA Recovered From Minute Amounts Of Serum Or Plasma"; March 1995; pp 45-51; XP004021069
JC	AE		Database Medline; US National Library of Medicine (NLM); Bethesda, MD, US; Lo et al.; "Presence Of Fetal DNA In Maternal Plasma And Serum"; AN (NLM) 97420079; XP002070361; See also Lancet, August 1997; 350 (9076) pp 485-487, England
JC	AF		Tsitologia; Vol. 37, No. 3; Kazakov et al.; "Extracellular DNA In The Blood Of Pregnant Women"; 1995; Institute of Cytology, Russian Academy Of Sciences, and Medical Academy Of Post Graduate Education, St. Petersburg; pp 1-8

EXAMINER	Jeanine Emerald	DATE CONSIDERED	March 10, 2000.

EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#9

In the **PATENT APPLICATION** of:	
	Lo et al.
Application No.:	09/380,696
Filed:	September 3, 1999
For:	NON-INVASIVE PRENATAL DIAGNOSIS
Group:	Not Yet Known
Examiner:	Not Yet Known

Our File: SHP-PT048

Date: November 2, 1999

REFUND REQUEST

Box 17
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. § 1.28(a) a refund of Four Hundred Seventy Four Dollars ($474) for the filing fee is respectfully requested. Enclosed herewith is a Declaration Supporting Claim for Small Entity Status By Small Business Concern.

This Refund Request is timely, being made prior to the first business day of the two month anniversary of the filing date on which the full filing was paid, 37 C.F.R. § 1.7.

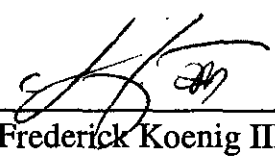
Applicant: Lo et al.
Application No.: 09/380,696

Please credit the refund to Deposit Account No. 22-0493 under our Order No. 725.

Two copies of this communication are enclosed.

Respectfully submitted,

Lo et al.

By 

C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

VOLPE and KOENIG, P.C.
400 One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/ras
Enclosures

March 6, 2000

C Frederick Koenig
Volpe and Koenig
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1617 John F Kennedy Boulevard
Philadelphia PA 19103

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

United States Receiving Office
United States International Searching Authority
United States International Preliminary Examining Authority
United States Designated Office
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Address: Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Dear Sir:

We regret to inform you that your request for refund dated 11/08/99 in the amount of $474.00 covering a fee for application serial number 09380696 cannot be authorized. Please refer to the box checked below.

(X) Small entity status fee not refundable. The time has expired for refund of this fee. A refund based on establishment of small entity status may only be obtained if a verified statement under 37 CFR 1.27 and a request for refund of the excess amount are filed within two months of timely payment of the full fee (37 CFR 1.28).

( ) Application or petition fee not refundable.. Money paid by actual mistake or in excess, such as payment not required by law, will be refunded; but a mere change of purpose after payment of money, as when a party desires to withdraw an application, an appeal or a request for oral hearing does not entitle the party to a refund 37 CFR 1.26). If any application is filed without the specification or drawing and the omission is not corrected within the period set, the application will be returned or otherwise disposed of. The fee, if submitted should include the $_____ handling fee (37 CFR 1.53).

( ) No refund is due. The charge of $ 260.00, is correct as filed for multiple dependent claims. PCT DO/EO does not use the substitute specification to change claims.

Any further questions concerning this refund, should be directed To PCT Tamala Holland at 703-305-5483.

Sincerely,

Tamala Holland /for/

Catherine Short
National Stage Supervisor

UNITED STAT DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/380,696	11/29/99	LO Y	SHP-PT048

HM22/0418

C FREDERICK KOENIG III
VOLPE & KOENIG
400 ONE PENN CENTER
1617 JOHN F KENNEDY BOULEVARD
PHILADELPHIA PA 19103

EXAMINER	
ENEWOLD, J	
ART UNIT	**PAPER NUMBER**
1655	9

DATE MAILED: 04/18/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/380,696	Applicant(s) LO ET AL.	
	Examiner Jeanine A Enewold	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

1)☒ Responsive to communication(s) filed on *November 29,1999, November 8, 1999* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

14) ☒ Notice of References Cited (PTO-892)
15) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
16) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6* .
17) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
18) ☐ Notice of Informal Patent Application (PTO-152)
19) ☐ Other: .

DETAILED ACTION

Priority

1. This application is a 371 of GB98/00690, filed March 4, 1998. This application also claims priority to GB9704444, filed March 4, 1997. However, claims 7-8, 17, 20-21, and 24 are not supported by GB9704444. Claims 7-8 are not supported by the GB9704444 document because although the document discloses sex determination and other polymorphisms which are present in the father, but not the mother, the disclosure does not describe either detecting DYS14 locus nor the SRY gene. Claim 17 is directed to variations of fetal DNA concentrations over the different stages of gestation, however, no mention of this difference was disclosed in the Great Britain document. Claims 20-21 are directed to specific concentrations of fetal DNA, which were not disclosed in the foreign priority document. Although the document discloses that "another potential application is the quantification of fetal nucleic acid in maternal serum or plasma", no specifics were provided (pg. 5). Finally, Claim 24 is not supported by the foreign document because no mention of clotting to extract serum and plasma is provided. Therefore, Claims 7-8, 17, 20-21, and 24 receive benefit of the GB98/00690 application filed March 4, 1999.

Drawings

2. The drawings are objected by the draftsman (see PTO 948).

Specification

3. This application does not contain an abstract of the disclosure as required by 37 CFR 1.72(b). An abstract on a separate sheet is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

4. Claims 1-5, 9-11 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The essential limitations of the claimed invention are directed to a method for detecting the presence of fetal DNA in maternal plasma in paternally-inherited non-Y sequences.

The specification teaches the detection of one paternally-inherited non-Y sequence, Rh-D gene. The specification teaches Rh-D genotyping from plasma from RhD negative pregnant women (pg. 20).

The art describes the detection of an expanded CGT trinucleotide repeats, in the DM kinase gene on chromosome 19, in the range of 50-4000 repeats in maternal serum of a pregnant women (Amicucci et al, February 2000, Clinical Chemistry, Vol 46, No. 2,

pages 301-302). Amicucci teaches sampling of plasma from pregnant women at 10 weeks of gestation to detect the expanded repeat present only in the father.

There is not adequate description for detection of the large genus of paternally-inherited non-Y sequences. The specification and the art only disclose two species within the scope of the genus: paternally inherited non-Y sequences. The general knowledge in the art concerning paternally inherited non-Y sequences does not provide any indication of how to detect any paternally inherited non-Y sequences based upon the teaches of two paternally inherited non-Y sequences. The two gene described are not representative of the genus of paternally inherited non-Y sequences. There is substantial variability among the species of nucleic acids encompassed in the scope of the claim because detection of paternally inherited non-Y sequences may include the detection of huge repeat expansions, like in the DM kinase gene, single gene mutations, deletions, and translocations. The specification has also not defined a structural feature for the detection of the paternally inherited non-Y sequences which would be common to all members of the genus that constitutes a substantial portion of the genus. Therefore, one of skill in the art would conclude that applicant was not in possession of the claimed detection of fetal DNA from a paternally inherited non-Y sequences because the description of only two members of this genus is not representative of the variable species within the genus and is insufficient to support the claims. Thus, the specification does not adequately provide a written description for a method for detecting paternally-inherited non-Y sequences.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

5. Claims 1-26 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for detecting the presence of paternally inherited fetal DNA in maternal plasma after 15 weeks of gestation wherein the fetal DNA is from the Y chromosome and for detecting the presence of the RhD gene in maternal plasma from an RhD negative pregnant women after 15 weeks gestation, does not reasonably provide enablement for a detection method performed on serum or plasma for detecting fetal nucleic acid in general at any time during pregnancy or associated with desiease phenotype in serum. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to us the invention commensurate in scope with these claims.

The claims are broadly drawn to a detection method performed on serum or plasma of a pregnant woman to detect any fetal DNA at any point in pregnancy.

The specification teaches fetal DNA has been detected in both serum and plasma. Table 2 and 3 show the quantification of fetal DNA in maternal serum and plasma in relation to the gestational age (pg. 33). The specifications teaches the detection of the Y-chromosome by markers to DYS14 locus and SRY gene. The specification teaches that plasma and serum samples were collected from 43 pregnant women with gestational ages

from 12 to 40 weeks (pg. 9, para. 1). Of the 30 male fetuses, detection of a Y-positive signal occurred in 24 plasma samples and only 21 serum samples (pg. 9,para. 1). The specification also teaches a RhD status determination from plasma of RhD-negative pregnant women (pg. 15 and Table 1, pg. 20).

The art teaches unpredictability in detecting fetal DNA in plasma before the 15th week of gestation, of detecting paternally inherited non-Y sequences, and the unpredictability of detecting fetal DNA in serum samples. Specifically, Lo et al (New England J. of Med. , Vol 339, No. 24, pages 1734-8, December 1998) teaches reliable results for fetal RhD status determination were obtainable from the 15th week of gestation and beyond in RhD negative women. Lo teaches that 7 of 9 fetus were positive on PCR testing for RhD genotyping (Table 1, pg. 1736). Lo teaches that two women with gestation ages of eight and nine weeks yielded false negative results (pg. 1735, col. 2, para. 6). Lo explicitly states "our data suggests that results of the RhD PCR test are reliable beginning in the second trimester" (pg. 1736, col. 2, para. 2). Additionally, Lo (Annals of Medicine, Vol. 31, NO. 5, pg. 308-312, Oct 1999) teaches "it is likely that future improvements in technology may allow more accurate diagnosis to be made and potentially extend the applicability of this method to the first trimester of pregnancy" (pg. 310, col. 2, para. 1) suggesting that the technology does not currently exist and may not have been conceived of as of yet what would be required to diagnose in the first trimester.

Moreover, the art teaches the detection of fetal DNA in maternal plasma for an expanded CGT trinucleotide repeats, in the DM kinase gene on chromosome 19, in the

range of 50-4000 repeats (Amicucci et al, February 2000, Clinical Chemistry, Vol. 46, No. 2, pages 301-302). Amicucci teaches sampling of plasma from pregnant women at 10 weeks of gestation to detect the expanded repeat present only in the father. Amicucci states "at present, this test seems appropriate only for monitoring paternally inherited expanded alleles" (pg. 302, para. 2). Additionally, Lo (Annals of Medicine, Vol 31, NO. 5, pg. 308-312, Oct 1999) states "the success of the detection of fetal-derived RhD gene in the plasma and serum of pregnant women opens up the possibility that a similar approach may be used for other single-gene disorders" (pg. 310, col. 2, para. 3). However, Lo has not taught single gene disorders other than RhD which may in fact use this technique. Furthermore, the RhD analysis was only shown to be succesful on RhD-negative women. The language of the paper is that of suggestion, and hypothesis rather than of evidence that this method works for these suggested single-gene disorders.

Furthermore, Lo (Annals of Medicine, Vol 31, NO. 5, pg. 308-312, Oct 1999) teaches increase amount of maternal DNA have been found in serum when compared with plasma (pg. 310, col. 1, para. 3). Further, the results "indicated that a higher maternal background is present when serum is used which may be detrimental for the detection of fetal DNA, especially when less sensitive detection methods are used"(pg. 310, col. 1, para. 3). Bianchi (Am. J. Hum. Genetics, Vol. 62, pg. 763-764, April 1998) teaches that the fractional concentration of fetal nucleic acid in serum was significantly less because of the increased amount of total DNA in serum (pg. 763, col. 1, para. 3). Bianchi moreover teaches that these results validate the results of Lo which showed that fetal DNA would be

reliably detected in as little of 10 microliters of maternal plasma. Furthermore, Bianchi states that "although fetal aneuploidy might be suggested by increased amounts of fetal DNA present in maternal plasma, cytogenetic confirmation using intact nuclei will ultimately be necessary (pg. 764, col. 1, para. 3). Bischoff et al (J. of the Society for Gynecologic Investigation, Vol. 6, No. 2, pages 64-69, Mar-April 1999) teaches detection of RhD in serum. However, Bischoff teaches that "our less than 100% detection efficiency probably reflects serum DNA purity, variable fetal DNA concentration in maternal serum, and DNA degradation caused by freezing and thawing of the serum samples" (pg. 67, col. 1).

Neither the specification nor the art provide guidance to overcome the unpredictability of detecting fetal DNA in plasma before the 15th week of gestation, of detecting paternally inherited non-Y sequences, and the unpredictability of detecting fetal DNA in serum samples. It would require undue experimentation for the ordinary artisan to practice the invention as broadly as claimed. The concentration of fetal DNA in maternal plasma at early stages of gestation appears to be low. Thus predictably detecting fetal DNA in maternal plasma samples before the 15th week of gestation is unpredictable and would require the ordinary artisan to enrich the fetal DNA in some manner which have not been described. In addition clinical studies would be required to determine the level of sensitivity of detection of paternally inherited sequences. Since, Amicucci explicitly states in his work as of February 2000, "at present, this test seems appropriate only for monitoring paternally inherited expanded alleles" (pg. 302, para. 2), it appears the sensitivity of the method can only detect huge expansions. Thus, detection of all paternally

inherited non-Y sequences would be unpredictable. While, the detection of paternally inherited non-Y sequences includes huge expansions, detection of single gene mutations which differed from mother to father, translocations, deletions would be unpredictable. Finally, the detection of fetal DNA in serum appears unpredictable based upon the teachings by Lo that the results "indicated that a higher maternal background is present when serum is used which may be detrimental for the detection of fetal DNA, especially when less sensitive detection methods are used"(pg. 310, col. 1, para. 3). Thus, the above analsysi demonstrates that the skilled artisan would be required to perform undue experimentation to make and use the invention as claimed.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> **The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.**

6. Claims 10 and 11 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 10 and 11 are indefinite over the recitation "such as", the phrase "such as" renders the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

Claim Rejections - 35 USC § 102

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

7. Claims 7 and 17 are rejected under 35 U.S.C. 102(a) as being anticipated by Lo (Lancet, August 1997).

It is noted that the authorship of the Lo et al. reference is distinct from the inventorship of the instant application and that this rejection may be overcome by the filing of a 132 Katz-type declaration. This rejection applies to the claims because as discussed previously these claims do not have foreign priority to the March 4, 1997 British patent application.

Lo et al. (herein referred to as Lo) teaches the detection of fetal DNA in maternal plasma and serum (abstract). Lo further teaches the detection of DYS14 from the Y chromosome (pg. 486, col. 1, para. 2)(limitations of Claim 7). Lo teaches that fetal DNA increases as gestation progresses (pg. 487, col. 1, para. 3)(limitations of Claim 17).

Conclusion

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Enewold whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Thursday from 7:00AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jeanine Enewold
April 12, 2000

LISA B. ARTHUR
PRIMARY EXAMINER
GROUP ~~1800~~ 1600

Notice of References Cited	Application/Control No. 09/380,696	Applicant(s)/Patent Under Reexamination LO ET AL.
	Examiner Jeanine A Enewold	Art Unit 1655
		Page 1 of 2

U.S. PATENT DOCUMENTS

*		DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	DOCUMENT SOURCE ** APS	OTHER
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NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title Date, Source, and Pertinent Pages)	DOCUMENT SOURCE ** APS	OTHER
☐	U	Lo et al "Presence of fetal DNA in maternal plasma and serum" Lancet, Vol 350, pages 485-487, August 1997.	☐	☐
☐	V	Lo "Fetal RhD genotyping from maternal plasma" Annals of Medicine, Vol 31, No. 5, pages 308-3012, Oct 1999.	☐	☐
☐	W	Bianchi "Fetal DNA in Maternal Plasma: The plot thickens and the placental barrier thins" Am. J. Hum. Genet. Vol 62, pages 763-764, April 1998.	☐	☐
☐	X	Lo et al "Prenatal Diagnosis of Fetal RhD status by molecular analysis of maternal plasma" New England J. of Med. Vol 339, No. 24, pages 1734-1738, Dec 1998.	☐	☐

*A copy of this reference is not being furnished with this Office action. (See Manual of Patent Examining Procedure, Section 707.05(a))
**APS encompasses any electronic search i.e. text, image, and Commercial Databases.

Notice of References Cited	Application/Control No. 09/380,696	Applicant(s)/Patent Under Reexamination LO ET AL.
	Examiner Jeanine A Enewold	Art Unit 1655
		Page 2 of 2

U.S. PATENT DOCUMENTS

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FOREIGN PATENT DOCUMENTS

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☐	T							☐	☐

NON-PATENT DOCUMENTS

*		DOCUMENT (Including Author, Title Date, Source, and Pertinent Pages)	DOCUMENT SOURCE ** APS	OTHER
☐	U	Anucucci et al "Prenatl diagnosis of Myotonic Dystrophy using fetal DNA obtained from maternal plasma" Clinical Chemistry, VOl 46, No. 2, pages 301-302, February 2000.	☐	☐
☐	V	Bischoff et al "Noninvasive Determination of Fetal RhD status using fetal DNA in Maternal Serum and PCR" J. of the Society for gynecologic investigation, Vol 6, NO. 2, apges 64-69, Mar-Aprl 2000.	☐	☐
☐	W		☐	☐
☐	X		☐	☐

*A copy of this reference is not being furnished with this Office action. (See Manual of Patent Examining Procedure, Section 707.05(a))
**APS encompasses any electronic search i.e. text, image, and Commercial Databases

Form PTO 948 (Rev. 8-98) U.S. DEPARTMENT OF COMMERCE - Patent and Trademark Office Application No. 9/380,696

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

The drawing(s) filed (insert date) 11-29-99 are:

A. ☐ approved by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☑ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 for the reasons indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawing must be sumitted according to the instructions on the back of this notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings: Black ink. Color.
   ____ Color drawings are not acceptable until petiton is granted. Fig(s) _____
   ____ Pencil and non black ink not permitted. Fig(s) _____
2. PHOTOGRAPHS. 37 CFR 1.84 (b)
   ____ 1 full-tone set is required. Fig(s) _____
   ____ Photographs not properly mounted (must use brystol board or photographic double-weight paper). Fig(s) _____
   ____ Foor quality (half-tone). Fig(s) _____
3. TYPE OF PAPER. 37 CFR 1.84(e)
   ____ Paper not flexible, strong, white, and durable. Fig(s) _____
   ____ Erasures, alterations, overwritings, interlineations, folds, copy machine marks not accepted. Fig(s) _____
   ____ Mylar, velum paper is not acceptable (too thin). Fig(s) _____
4. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:
   ____ 21.0 cm by 29.7 cm (DIN size A4)
   ____ 21.6 cm by 27.9 cm (8 1/2 x 11 inches)
   ____ All drawing sheets not the same size. Sheet(s) _____
   ____ Drawings sheets not an acceptable size. Fig(s) _____
5. MARGINS. 37 CFR 1.84(g): Acceptable margins:

   Top 2.5 cm Left 2.5cm Right 1.5 cm Bottom 1.0 cm
   SIZE: A4 Size
   Top 2.5 cm Left 2.5 cm Right 1.5 cm Bottom 1.0 cm
   SIZE: 8 1/2 x 11
   Margins not acceptable. Fig(s) _____
   _____ Top (T) _____ Left (L)
   _____ Right (R) _____ Bottom (B)
6. VIEWS. 37 CFR 1.84(h)
   REMINDER: Specification may require revision to correspond to drawing changes.
   Partial views. 37 CFR 1.84(h)(2)
   ____ Brackets needed to show figure as one entity. Fig(s) _____
   ____ Views not labeled separately or properly. Fig(s) 4
   ____ Enlarged view not labeled separetely or properly. Fig(s) _____
7. SECTIONAL VIEWS. 37 CFR 1.84 (h)(3)
   ____ Hatching not indicated for sectional portions of an object. Fig(s) _____
   ____ Sectional designation should be noted with Arabic or Roman numbers. Fig(s) _____
8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)
   ____ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____
9. SCALE. 37 CFR 1.84(k)
   ____ Scale not large enough to show mechanism without crowding when drawing is reduced in size to two-thirds in reproduction. Fig(s) _____
10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(i)
    ____ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (poor line quality). Fig(s) _____
11. SHADING. 37 CFR 1.84(m)
    ____ Solid black areas pale. Fig(s) _____
    ____ Solid black shading not permitted. Fig(s) _____
    ____ Shade lines, pale, rough and blurred. Fig(s) _____
12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)
    ____ Numbers and reference characters not plain and legible. Fig(s) _____
    ____ Figure legends are poor. Fig(s) _____
    ____ Numbers and reference characters not oriented in the same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____
    ____ English alphabet not used. 37 CFR 1.84(p)(2) Figs _____
    ____ Numbers, letters and reference characters must be at least .32 cm (1/8 inch) in height. 37 CFR 1.84(p)(3) Fig(s) _____
13. LEAD LINES. 37 CFR 1.84(q)
    ____ Lead lines cross each other. Fig(s) _____
    ____ Lead lines missing. Fig(s) _____
14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(t)
    ____ Sheets not numbered consecutively, and in Arabic numerals beginning with number 1. Sheet(s) _____
15. NUMBERING OF VIEWS. 37 CFR 1.84(u)
    ____ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____
16. CORRECTIONS. 37 CFR 1.84(w)
    ____ Corrections not made from prior PTO-948 dated _____
17. DESIGN DRAWINGS. 37 CFR 1.152
    ____ Surface shading shown not appropriate. Fig(s) _____
    ____ Solid black shading not used for color contrast. Fig(s) _____

COMMENTS

REVIEWER JC DATE 1-3-00 TELEPHONE NO. 703305-8478

ATTACHMENT TO PAPER NO. 9

10/13 1.Wms 9/29/00

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:		
	Lo et al.	
Application No.:	09/380,696	Our File: SHP-PT048
Filed:	November 29, 1999	Date: September 15, 2000
For: NON-INVASIVE PRENATAL DIAGNOSIS		
Group:	1655	
Examiner:	J. Enewold	

REPLY PURSUANT TO 37 C.F.R. § 1.111

Commissioner for Patents
Washington, D.C. 20231

Sir:

This Reply is responsive to the Examiner's Action dated April 18, 2000. The Applicants respectfully request that the Application be amended as follows:

IN THE ABSTRACT

Please delete the abstract from the face sheet of the PCT published application and substitute therefor the ABSTRACT submitted herewith on a separate sheet.

IN THE DRAWINGS

A proposed revision to separately identify the individual graphs of Figure 4 as indicated in red on the attached sheet is submitted herewith.

Applicant: Lo et al.
Application No.: 09/380,696

IN THE SPECIFICATION

On page 8, line 14, please delete "Figure 4 shows" and insert -- Figures 4a-4l show --.

On page 29, line 2, please delete "fig. 4" and insert -- Figures 4a-4l --.

On page 31, line 14, please delete "fig. 4" and insert -- Figures 4a-4l --.

On page 34, line 19, please delete "Figure 4" and insert -- Figures 4a-4l --.

IN THE CLAIMS

Please amend the following claims:

9 ~~N~~. (Amended) The method according to claim ~~1~~ 8 wherein the non-Y sequence is a blood group antigen gene [such as the Rhesus D gene].

B1

~~N~~10. (Amended) The method according to claim ~~9~~ 8, wherein the non-Y sequence is a gene which confers a disease phenotype in the foetus [, such as the Rhesus D gene].

Please add the following new claims:

B2

~~26~~27. The method according to claim ~~N~~9, wherein the blood group antigen gene is the rhesus D gene.

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B

CONT B2

28. The method according to claim 10, wherein the gene is the rhesus D gene.--

REMARKS

The drawings have been objected to because the views of Figure 4 were not labeled separately. Approval of the proposed drawing changes as indicated on the attached sheet is requested. No new matter has been added.

Applicants have amended the specification to conform with the drawing amendment. An abstract on a separate sheet has been provided as required. No new matter has been added.

Claims 1-26 are pending in the application. A priority claim to GB9704444 (hereinafter "the priority document"), filed March 4, 1997, was objected to with respect to Claims 7-8, 17, 20-21 and 24. Claims 1-26 were rejected under the first paragraph of 35 U.S.C. §112. Claims 10-11were rejected under the second paragraph of 35 U.S.C. §112. Claims 7 and 17 were rejected under 35 U.S.C. §102(a) as being anticipated by Lo (Lancet, August 1997).

Applicants respectfully traverse the Examiner's priority objection and anticipation rejection of claims 7 and 17 over Lo (Lancet, August 1997). With respect to claims 8, 20-21 and 24, the objection is not ripe since no rejection over intervening art has been made. With

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respect to the Examiner's assertion that claim 7 is not supported by the priority document because it includes no reference to detecting DYS14, Applicants respectfully disagree. Page 8, lines 2-5 of the priority document explicitly refer to amplification of a single copy of Y sequence DYS14.

With respect to claim 17, the Examiner asserts that the priority document does not disclose variations of fetal DNA concentrations over the different stages of gestation. Applicants respectfully disagree. Applicants submit that the priority document clearly describes that variations in the quantity of foetal DNA may occur in some pregnancy-associated conditions such as pre-eclampsia. Specifically, page 2, lines 24-27, specifically refers to differing amounts of foetal DNA being present in the maternal serum or plasma. One skilled in the art would readily understand that this would refer to a variation of foetal DNA concentration at a particular stage of gestation. Further, at the priority filing date, one skilled in the art would have also been aware that foetal DNA generally shows a variation over the course of a pregnancy. In order to monitor whether there is a higher or lower level of foetal DNA compared to normal, it would be desirable to make a comparison with a sample from a similar stage of gestation.

Although the priority document does not include identical claims as now on file, Applicants respectfully submit that the disclosure of the priority document, as read by one skilled in the art, clearly encompasses rejected claims 7 and 17. Accordingly, the §102 rejection based on Lo (Lancet, August 1997) is traversed as not being prior art to these claims.

The rejection of Claims 1-5 and 9-11 under the first paragraph of 35 U.S.C. §112, as containing subject matter which was not adequately described in the specification, is respectfully traversed. The Examiner contends that there is not adequate description for the detection of the large genus of paternally-inherited non-Y sequences. Although, as noted by the Examiner, there is substantial variability among the species of nucleic acids encompassed in the scope of the claim, Applicants submit that one skilled in the art is aware of a variety of techniques which might be used to detect different nucleic acid species. For example, there are numerous techniques which might be used to detect repeat expansions, single gene mutations, deletions or translocations. These techniques are a matter of routine for one skilled in the art for the analysis of DNA.

Further, the invention does not rely on the identification of any specific paternally-inherited non-Y sequences. The invention resides in the identification of foetal DNA in a serum or plasma sample. One skilled in the art could take advantage of the present

application describing the presence of foetal DNA in the plasma or serum and apply it to the detection of paternally-inherited non-Y sequences in addition to those which are described. For example, the Examiner has referred to an article by Amicucci et al. which clearly describes detection of an expanded repeat. The Amicucci et al. article clearly demonstrates that the technique as described in the present application may be readily applied to the detection of repeat sequences.

Additionally, Applicants refer the Examiner to a number of other documents which describe analysis of foetal DNA in maternal plasma or serum. Attached herewith are copies of Pertl et al. *Human Genetics* 106(1) - 45-49, 2000 (Abstract), Tang et al. *Clinical Chemistry* 45, 11;1999; 2033-2035, Smid et al. *Clinical Chemistry* 45, 8; 1999; 1570-1572 and Chen et al. *Prenat Diagn* 2000, 20; 355-357. Each of these articles provides an example of the application of the general technique described in the present application to specific sequences. Each of these articles refers to the work done by the inventors of the present application disclosed in Lo et al. In particular, these articles refer to Lo et al. where it describes detection of foetal DNA in maternal plasma and serum and describes the technique to a variety of different sequences. Moreover, the articles cited above demonstrate that microsatellite alleles which differ by a very small number of nucleotides between the mother and baby, that is by 2 base pairs, are detectable using the technology described in the present

application. Microsatellites are essentially polymorphic pieces of DNA, which are different between different individuals by virtue of insertions or deletions of a small number of base pairs. The paper by Chen et al. describes the successful diagnosis of a paternally-inherited reciprocal translocation.

Additionally, there are numerous types of mutations that might be detected, in accordance with the present invention. The Examiner has discussed whether the technique is applicable for detecting small differences between the mother and foetus and has highlighted three categories, namely, single gene mutations, deletions, and translocations. The attached articles clearly demonstrate that a wide variety of different polymorphisms may be detected in accordance with present application. Applicants submit that there is sufficient description in that the key features of the claimed technique have been described in the Application, and, in particular, one skilled in the art is instructed to use maternal plasma or serum for the detection of foetal DNA. Although there are a wide variety of different types of polymorphisms which could be detected in connection with the present application, such polymorphisms and techniques for analysis of DNA are simply a matter of routine for one skilled in the art. Therefore, it is not necessary for the Applicants to set out each of the many ways in which DNA might be analyzed. The description is sufficient simply by instructing one skilled in the art to carry out a suitable analysis. The additional documents, attached

hereto, clearly demonstrate that one skilled in the art is readily able to apply the teachings of the present application to any one of the well known techniques for detection of DNA with a view to analysis of foetal DNA in paternal plasma or serum.

Applicants respectfully traverse the rejection of Claims 1-26 under the first paragraph 35 U.S.C. §112, on the basis of lack of enablement for a general detection method performed on serum or plasma for detecting fetal nucleic acid at any time during pregnancy or associated with disease phenotype and serum. The Examiner refers to Lo et al. (*New England J. of Med.*, Vol. 339, No. 24, pages 1734-8) and suggests that the claims are only enabled with respect to detecting the presence of paternally-inherited foetal DNA in maternal plasma after 15 weeks of gestation. The Examiner has indicated that there is unpredictability in detecting foetal DNA in plasma before the fifteenth week of gestation. However, Applicants respectfully submit that the specification is enabled across the scope of the breadth of the claim for a detection method performed on serum or plasma of pregnant women to detect any foetal DNA during the course of pregnancy. Although the article cited by Examiner suggests that reliable results for foetal RHD status can be determined from the fifteenth week of gestation, the paper nevertheless demonstrates that testing prior to 15 weeks of gestation is already useful.

The Examiner has cited some of the Applicants' own comments in the article of Lo et al., *Annals of Medicine*, Volume 31, 5: 1999; 308-312. As with all technologies, it can be expected that improvements in the technology may arise. For example, it is likely that improvements will be made to enhance sensitivity of the techniques. However, this is not to say that the techniques can not be used as a diagnostic method across the scope of the claims. Clearly, the statements quoted by the Examiner in the *Annals of Medicine* cannot be seen as a suggestion that the technique does not in itself work effectively.

With respect to the dividing line of 15 weeks, the article by Lo et al. referred to by the Examiner merely states that for RHD, PCR tests are reliable beginning in the second trimester. This is not to say that such tests can not be useful when carried out before the second trimester. For example, if a potential problem were highlighted in a test carried out before the second trimester, this problem could be used as part of a diagnosis such as to identify women who require close monitoring in later stages, for example to confirm a provisional diagnosis. Thus, it may be possible to identify such things as a foetus at risk of foetal hemolytic disease before 15 weeks of pregnancy and highlight that pregnancy for enhanced surveillance.

There are also numerous papers showing that the technology can be used prior to the 15th week of gestation. In Lo et al., *American Journal of Human Genetics* of 1998, 62; 768-

B

775, the authors show that foetal DNA can be detected from maternal serum at the seventh week of gestation. Amicucci et al. demonstrates that the technology can be used at the tenth week of gestation. Smid et al., *Clinical Chemistry* 1999, 45;1570-1572 demonstrates that the method is applicable between the seventh and fourteenth weeks of gestation.

Additionally, the Examiner also refers to a number of papers as suggesting that there are potential problems with the technique and that to a certain extent the claims are based on hypothesis. As highlighted above, the present invention results in the new identification that foetal DNA is present in maternal plasma or serum. Many of the points highlighted by the Examiner would be considered to be a matter of routine experimentation to one skilled in the art of DNA detection, to identify the most appropriate technique for a particular required diagnosis. The person skilled in the art has a broad range of techniques available for the detection of DNA in a sample. Thus, one skilled in the art, equipped with the teaching of the present patent application, would be readily able to overcome any such potential problems mentioned by the Examiner. Indeed, there is much literature, such as the articles referred to above, which demonstrates that the technique has been successfully applied to other sequences.

The Examiner further suggests that there may be a problem in connection with using material serum and that increased amount of maternal DNA can be found. The Examiner quotes Lo et al.:

> The results indicated that a higher maternal background is present when serum is used which may be detrimental for the detection of foetal DNA, especially when less sensitive detection methods are used.

Applicants submit that one skilled in the art would understand simply that a higher maternal background may be present where serum is used, and that it may be preferable to use a more sensitive detection method. However, as highlighted above, this statement does not in any way suggest that the technique can not be used. The statement merely suggests that the technique should be optimized given the particular circumstances. This is simply a straightforward matter of application of an appropriate DNA detection method.

The Examiner has highlighted some problems in using serum samples, highlighted by Bischoff et al. However, one skilled in the art would simply take appropriate action to avoid the specific problems highlighted in this article. The article does not suggest that the method would in any way not work simply because serum DNA was being used. In any event, there are a number of papers which have used maternal serum reliability for detection of foetal DNA, namely, Lo et al. *American Journal of Human Genetics* Supra, Lo et al., *Clinical Chemistry* 1999, 45;184-188 (abstract attached).

In summary, the, various documents cited by the Examiner do not suggest that the present technique would not be successful. Improvement of the process or selection of the most appropriate of DNA analysis is simply a matter of routine experimentation which would be carried out by one skilled in the art based on the readily available techniques of DNA detection.

With respect to the rejection of Claims 10 and 11 under 35 U.S.C. §112, Applicants have amended these claims to delete the phrase "such as" objected to by the Examiner. New dependent claims 27 and 28 have been added. Applicants believe these claims are now in condition for allowance.

It is respectfully submitted that the pending claims as amended are now in condition for allowance. Reconsideration, approval of the drawing amendment, and allowance are respectfully requested.

Applicant: Lo et al.
Application No.: 09/380,696

Respectfully submitted,

Lo et al.

By _____
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(215) 568-6400

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Suite 400, One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/JMO/dag

Attachments: Pertl et al. *Human Genetics*
Tang et al. *Clinical Chemistry*
Smid et al. *Clinical Chemistry*
Chen et al. *Prenat Diagn*
Lo et al., *American Journal of Human Genetics*
Lo et al., *Clinical Chemistry*

Enclosures (2)

B

ABSTRACT

The invention relates to a detection method performed on a maternal serum or plasma sample from a pregnant female, which method comprises detecting the presence of a nucleic acid of foetal origin in the sample. The invention enables non-invasive prenatal diagnosis including for example sex determination, blood typing and other genotyping, and detection of pre-eclampsia in the mother.

Volpe and Koenig, P.C. Revision of

PTO/SB/17 (12/99)
Approved for use through 09/30/2000. OMB 0651-0032
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FEE TRANSMITTAL for FY 2000

Patent fees are subject to annual revision.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12. See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT ($) $208.00

Complete if Known	
Application Number	09/380,696
Filing Date	November 29, 1999
First Named Inventor	Lo et al.
Examiner Name	Enewold, J.
Group / Art Unit	1655
Attorney Docket No.	SHP-PT048

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge the fees indicated hereon:

Deposit Account Number: 22-0493

Deposit Account Name: Volpe and Koenig, P.C.

☒ Charge Any Deficiency or Credit Any Overpayment in the Total Fees Associated with this Communication

Our Order No. 1822

2. ☒ **Payment Enclosed:** ☒ Check ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	690	201	345	Utility filing fee	
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	690	208	345	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below	Fee Paid
Total Claims	28	- 20** =	2	X	$9.00	= $18.00
Independent Claims	3	- 3 ** =	0	X	$39.00	= 0
Multiple Dependent						=

**or number previously paid, if greater; For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 18.00

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	$190
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	690	246	345	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	690	249	345	For each additional invention to be examined (37 CFR § 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 190

SUBMITTED BY				Complete *(if applicable)*	
Name (Print/Type)	C. Frederick Koenig III, Esquire	*Registration No. (Attorney/Agent)*	29,662	*Telephone*	215-568-6400
Signature				*Date*	9/15/00

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/380,696
Filing Date	November 29, 1999
First Named Inventor	Lo et al.
Group Art Unit	1655
Examiner Name	Enewold, J.
Total Number of Pages in This Submission: 20	Attorney Docket Number: SHP-PT048

ENCLOSURES *(check all that apply)*

- [x] Fee Transmittal Form
  - [x] Fee Attached
- [x] Amendment / Response
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [x] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition Routing Slip (PTO/SB/69) and Accompanying Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Small Entity Statement
- [ ] Request for Refund
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Additional Enclosure(s) *(please identify below)*:

Attachments (6 Articles); Marked Up Drawing sheets (2 pgs.); Abstract (1 pg.)

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	C. Frederick Koenig III, Esquire Reg. No. 29,662 VOLPE and KOENIG, P.C.
Signature	
Date	9/15/00

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on this date: Sept. 15, 2000

Typed or printed name	C. Frederick Koenig III, Esquire		
Signature		Date	9/15/00

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Fig.4.

#11

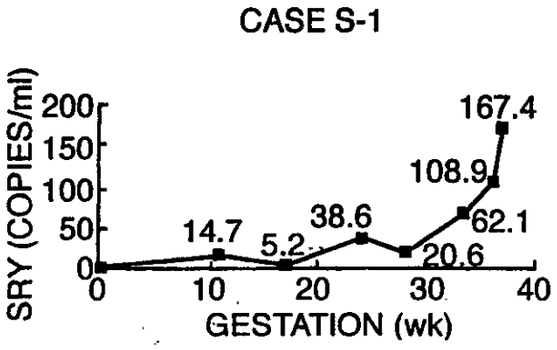

Fig. 4a

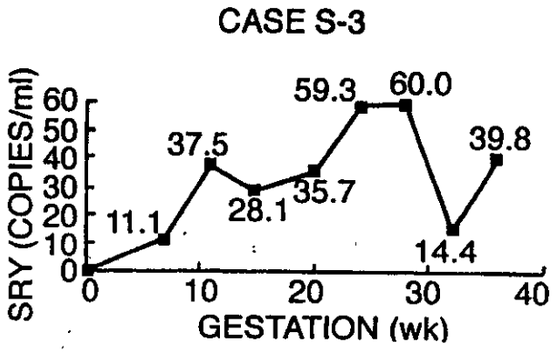

Fig. 4b

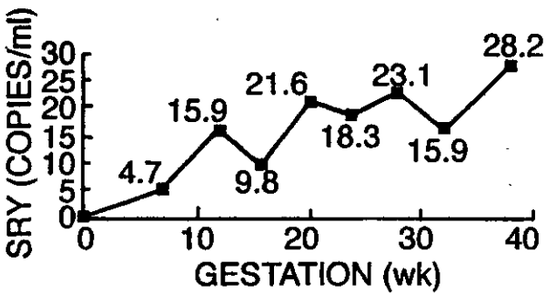

Fig. 4c

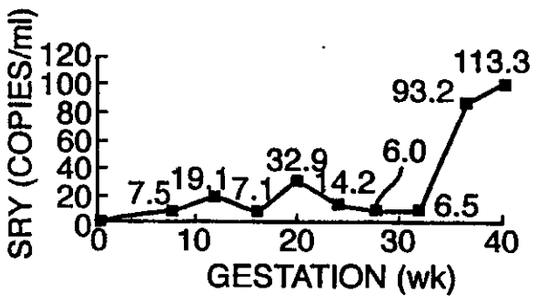

Fig. 4d

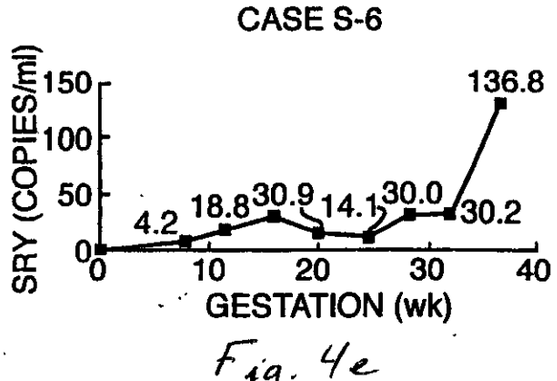

Fig. 4e

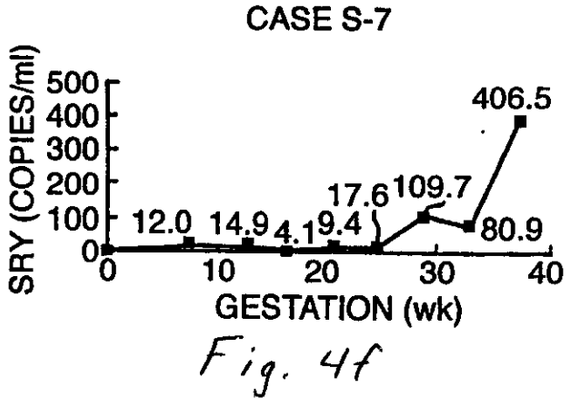

Fig. 4f

Fig.4(Cont.)

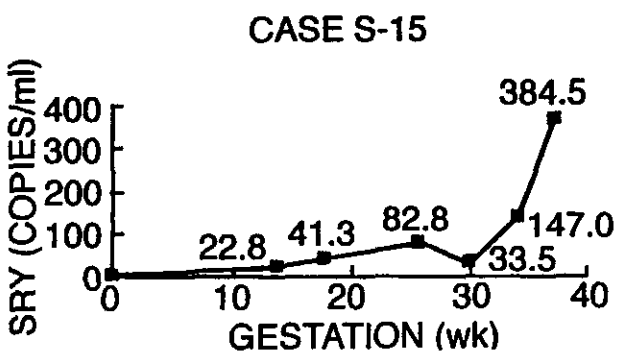

Fig. 4g

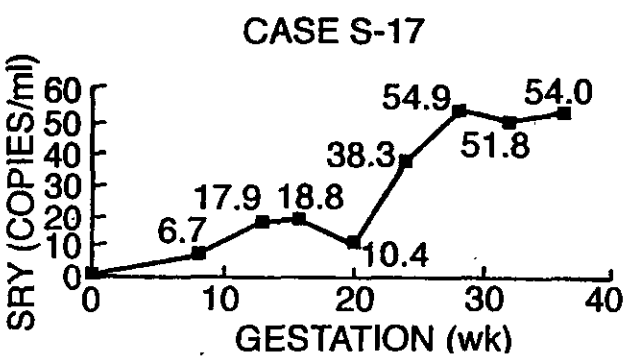

Fig. 4h

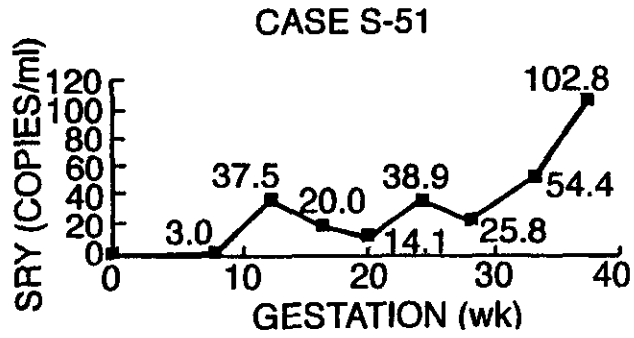

Fig. 4i

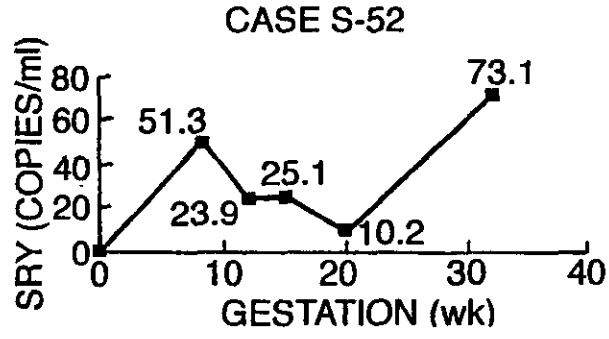

Fig. 4j

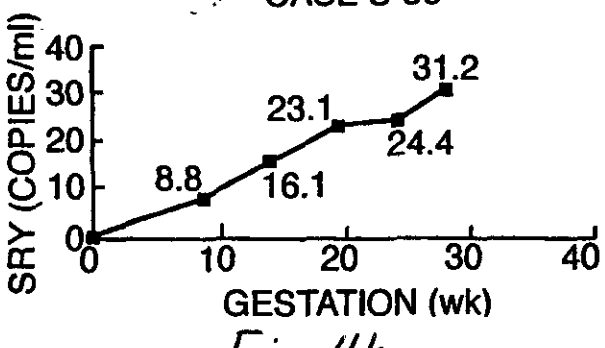

Fig. 4k

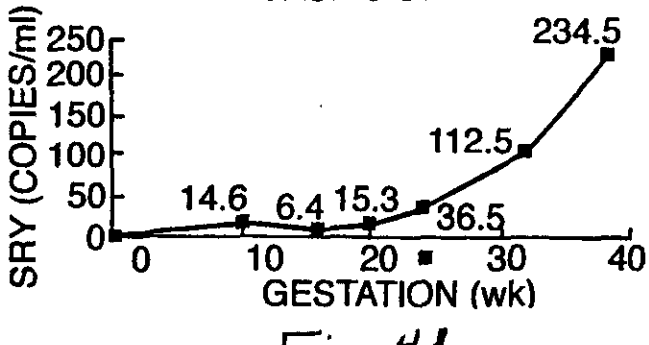

Fig. 4l

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/380,696	11/29/99	LO Y	SHP-PT048

HM22/1102

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EXAMINER	
GOLDBERG, I	
ART UNIT	**PAPER NUMBER**
1655	12

DATE MAILED: 11/02/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)

U.S G.P.O. 2000 ; 465-188/25266

1- File Copy

Office Action Summary	Application No.	Applicant(s)	
	09/380,696	LO ET AL.	
	Examiner	Art Unit	
	Jeanine A Enewold Goldberg	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U S.C. § 133).

Status

1)☒ Responsive to communication(s) filed on *22 September 2000* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-28* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-28* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

15) ☐ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other:

DETAILED ACTION

1. This action is in response to the papers filed September 22, 2000. Currently, claims 1-28 are pending. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow. This action is made FINAL.

2. Any objections and rejections not reiterated below are hereby withdrawn.

Maintained Rejections

Priority

3. This application is a 371 of GB98/00690, filed March 4, 1998. This application also claims priority to GB9704444, filed March 4, 1997. However, claims 7-8, 17, 20-21, and 24 are not supported by GB9704444. Claims 7-8 are not supported by the GB9704444 document because although the document discloses sex determination and other polymorphisms which are present in the father, but not the mother, the disclosure does not describe either detecting DYS14 locus nor the SRY gene. Claim 17 is directed to variations of fetal DNA concentrations over the different stages of gestation, however, no mention of this difference was disclosed in the Great Britain document. Claims 20-21 are directed to specific concentrations of fetal DNA, which were not disclosed in the foreign priority document. Although the document discloses that "another potential application is the quantification of fetal nucleic acid in maternal serum or plasma", no specifics were provided (pg. 5). Finally, Claim 24 is not supported by the foreign document because no mention of clotting to extract serum and plasma is

provided. Therefore, Claims 7-8, 17, 20-21, and 24 receive benefit of the GB98/00690 application filed March 4, 1999.

Response to Arguments

The response traverses the rejection. The response asserts that Claim 7 and 17 are supported by the specification of GB9704444, filed March 4, 1997, and thus should receive priority. This argument has been reviewed. With respect to Claim 7, the examiner acknowledges the document does refer to DYS14 and agrees that Claim 7 should receive benefit of March 1997.

However, with regards to Claim 17, the response asserts that the GB9704444, filed March 4, 1997 supports variations of fetal DNA concentrations over the different stages of gestation. The response states that "on skilled in the art would have also been aware that fetal DNA generally shows a variation over the course of a pregnancy. Further, the response states that "it would be desirable to make a comparison with a sample from a similar stage of gestation". This argument has been reviewed, but is not convincing because the priority document states "detection and monitoring of pregnancy-associated conditions such as pre-eclampsia which may result in differing amounts of foetal DNA being present in the maternal serum or plasma" (pg. 2, lines 24-27). This statement while proposing that variation occurs, does not provide any specific evidence that variations in fact exist, nor provides the variations from normal as stated in Claim 17. Secondly, based upon the inventive nature presumed for the invention, the skilled artisan would not have been aware that fetal DNA existed in the serum/plasma, and thus would not have "been aware that foetal DNA shows a variation over the course

of pregnancy". Finally, it is acknowledged that "a comparison" would be desirable, however, the comparison was not performed in the foreign document.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

4. Claims 1-26 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for detecting the presence of paternally inherited fetal DNA in maternal plasma after 15 weeks of gestation wherein the fetal DNA is from the Y chromosome and for detecting the presence of the RhD gene in maternal plasma from an RhD negative pregnant women after 15 weeks gestation, does not reasonably provide enablement for a detection method performed on serum or plasma for detecting fetal nucleic acid in general at any time during pregnancy or associated with disease phenotype in serum. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to us the invention commensurate in scope with these claims.

The claims are broadly drawn to a detection method performed on serum or plasma of a pregnant woman to detect any fetal DNA at any point in pregnancy.

The specification teaches fetal DNA has been detected in both serum and plasma. Table 2 and 3 show the quantification of fetal DNA in maternal serum and plasma in relation to the gestational age (pg. 33). The specifications teaches the

detection of the Y-chromosome by markers to DYS14 locus and SRY gene. The specification teaches that plasma and serum samples were collected from 43 pregnant women with gestational ages from 12 to 40 weeks (pg. 9, para. 1). Of the 30 male fetuses, detection of a Y-positive signal occurred in 24 plasma samples and only 21 serum samples (pg. 9,para. 1). The specification also teaches a RhD status determination from plasma of RhD-negative pregnant women (pg. 15 and Table 1, pg. 20).

The art teaches unpredictability in detecting fetal DNA in plasma before the 15th week of gestation, of detecting paternally inherited non-Y sequences, and the unpredictability of detecting fetal DNA in serum samples. Specifically, Lo et al (New England J. of Med. , Vol. 339, No. 24, pages 1734-8, December 1998) teaches reliable results for fetal RhD status determination were obtainable from the 15th week of gestation and beyond in RhD negative women. Lo teaches that 7 of 9 fetus were positive on PCR testing for RhD genotyping (Table 1, pg. 1736). Lo teaches that two women with gestation ages of eight and nine weeks yielded false negative results (pg. 1735, col. 2, para. 6). Lo explicitly states "our data suggests that results of the RhD PCR test are reliable beginning in the second trimester" (pg. 1736, col. 2, para. 2). Additionally, Lo (Annals of Medicine, Vol. 31, NO. 5, pg. 308-312, Oct 1999) teaches "it is likely that future improvements in technology may allow more accurate diagnosis to be made and potentially extend the applicability of this method to the first trimester of pregnancy" (pg. 310, col. 2, para. 1) suggesting that the technology does not currently

exist and may not have been conceived of as of yet what would be required to diagnose in the first trimester.

Moreover, the art teaches the detection of fetal DNA in maternal plasma for an expanded CGT trinucleotide repeats, in the DM kinase gene on chromosome 19, in the range of 50-4000 repeats (Amicucci et al, February 2000, Clinical Chemistry, Vol. 46, No. 2, pages 301-302). Amicucci teaches sampling of plasma from pregnant women at 10 weeks of gestation to detect the expanded repeat present only in the father. Amicucci states "at present, this test seems appropriate only for monitoring paternally inherited expanded alleles" (pg. 302, para. 2). Additionally, Lo (Annals of Medicine, Vol. 31, NO. 5, pg. 308-312, Oct 1999) states "the success of the detection of fetal-derived RhD gene in the plasma and serum of pregnant women opens up the possibility that a similar approach may be used for other single-gene disorders" (pg. 310, col. 2, para. 3). However, Lo has not taught single gene disorders other than RhD which may in fact use this technique. Furthermore, the RhD analysis was only shown to be successful on RhD-negative women. The language of the paper is that of suggestion, and hypothesis rather than of evidence that this method works for these suggested single-gene disorders.

Furthermore, Lo (Annals of Medicine, Vol. 31, NO. 5, pg. 308-312, Oct 1999) teaches increase amount of maternal DNA have been found in serum when compared with plasma (pg. 310, col. 1, para. 3). Further, the results "indicated that a higher maternal background is present when serum is used which may be detrimental for the detection of fetal DNA, especially when less sensitive detection methods are used"(pg.

310, col. 1, para. 3). Bianchi (Am. J. Hum. Genetics, Vol. 62, pg. 763-764, April 1998) teaches that the fractional concentration of fetal nucleic acid in serum was significantly less because of the increased amount of total DNA in serum (pg. 763, col. 1, para. 3). Bianchi moreover teaches that these results validate the results of Lo which showed that fetal DNA would be reliably detected in as little of 10 microliters of maternal plasma. Furthermore, Bianchi states that "although fetal aneuploidy might be suggested by increased amounts of fetal DNA present in maternal plasma, cytogenetic confirmation using intact nuclei will ultimately be necessary (pg. 764, col. 1, para. 3). Bischoff et al (J. of the Society for Gynecologic Investigation, Vol. 6, No. 2, pages 64-69, Mar-April 1999) teaches detection of RhD in serum. However, Bischoff teaches that "our less than 100% detection efficiency probably reflects serum DNA purity, variable fetal DNA concentration in maternal serum, and DNA degradation caused by freezing and thawing of the serum samples" (pg. 67, col. 1).

Neither the specification nor the art provide guidance to overcome the unpredictability of detecting fetal DNA in plasma before the 15th week of gestation, of detecting paternally inherited non-Y sequences, and the unpredictability of detecting fetal DNA in serum samples. It would require undue experimentation for the ordinary artisan to practice the invention as broadly as claimed. The concentration of fetal DNA in maternal plasma at early stages of gestation appears to be low. Thus predictably detecting fetal DNA in maternal plasma samples before the 15th week of gestation is unpredictable and would require the ordinary artisan to enrich the fetal DNA in some manner which have not been described. In addition clinical studies would be required to

determine the level of sensitivity of detection of paternally inherited sequences. Since, Amicucci explicitly states in his work as of February 2000, "at present, this test seems appropriate only for monitoring paternally inherited expanded alleles" (pg. 302, para. 2), it appears the sensitivity of the method can only detect huge expansions. Thus, detection of all paternally inherited non-Y sequences would be unpredictable. While, the detection of paternally inherited non-Y sequences includes huge expansions, detection of single gene mutations which differed from mother to father, translocations, deletions would be unpredictable. Finally, the detection of fetal DNA in serum appears unpredictable based upon the teachings by Lo that the results "indicated that a higher maternal background is present when serum is used which may be detrimental for the detection of fetal DNA, especially when less sensitive detection methods are used"(pg. 310, col. 1, para. 3). Thus, the above analysis demonstrates that the skilled artisan would be required to perform undue experimentation to make and use the invention as claimed.

Response to Arguments

The response traverses the rejection. The response asserts that specification is enabling across the scope of the breadth of the claim for detection method over the course of pregnancy. The response asserts that "the paper demonstrates that testing prior to 15 weeks of gestation is already useful". This argument has been reviewed but is not convincing because the art teaches that "noninvasive fetal RhD genotyping can be performed rapidly and reliably with the use of maternal plasma beginning in the second trimester of pregnancy" (abstract). The paper teaches that "plasma samples

from two women in the first trimester of pregnancy who were carrying RhD-positive fetuses, with gestational ages of eight and nine weeks, yielded false negative results". The paper explains "the results for the two first-trimester samples which were false negative, presumably because of the low concentration of fetal DNA in maternal plasma at that time" (pg. 1736, col. 2). The paper illustrates that amplification is required for sensitivity of the PCR analysis for the detection of RhD DNA, such that with 25 or fewer amplification cycles showed no intensity of fluorescence of study (Figure 1). The paper also illustrates that different weeks of gestation are detectable after different numbers of cycling (Figure 2). The teachings in the specification support these results such that the concentration of SRY in early pregnancy and late pregnancy differ substantially. For example, in early pregnancy plasma an average of 25.4 copies/ml are found while 292.2 copies/ml are found in late pregnancy. Similarly, in early pregnancy serum an average of 28.7 copies/ml are found while 342.1 copies/ml are found in late pregnancy. Which adds to the unpredictability of detecting fetal DNA in maternal serum/plasma prior 15 weeks of gestation and without any amplification.

Secondly, the response asserts that the comments found in Lo et al (Annals of Medicine, Vol. 31, No. 5, pg. 308-312, 1999) regarding applicability of the method for the first trimester, is not to say that the techniques can not be sued as a diagnostic method across the scope of the claims. This argument has been reviewed but is not convincing because the reference was cited to support the position that predictable detection prior to 15 weeks of gestation is unpredictable. The statement by Lo "it is likely that future improvements in technology may allow more accurate diagnosis to be

made and potentially extend the applicability of this method to the first trimester of pregnancy" indicates that currently the method is not applicable for the first trimester and even with the technological improvements, the accurate detection within the first trimester is unpredictable.

Thirdly, the response asserts that the statement that PCR tests are reliable beginning in the second trimester does not say that such tests can not be useful when carried out before the second trimester. This argument has been reviewed but is not convincing because the problem of detection prior to the second trimester appears to be sensitivity. The instant claims are not directed to a PCR or amplification method such that this step is required. Nevertheless, if the problem as stated by Lo et al (New England J. of Medicine) is detection of low concentration, it is likely that method contains false negatives. The response appears to be discussing false positives in which a "potential problem" may be highlighted. However, it seems as though the lack of detection of the nucleic acid would present more of a problem leading to the unpredictability. With regard to the three articles cited in the response that support the detection in the first trimester, each of these articles require that an amplification step is performed such that this detection is plausible, such that it appears that an amplification step is a critical feature of the invention. Smid provides different amplifications and illustrates that false-positive results occur.

In conclusion, based upon the remarks and arguments presented, it remains unpredictable to detect the presence of a nucleic acid of foetal origin in the sample prior to 15 weeks of gestation as provided above. Further, the claims remain broadly drawn

to the detection of nucleic acids of fetal origin, however, the detection of a maternally inherited nucleic acid from the fetus is unpredictable. The specification explicitly states that "the method of the invention can be applied to the detection of any paternally-inherited sequences which are not possessed by the mother" (pg. 4, lines 5-7). As stated in numerous of the papers the concentrations of fetal DNA in maternal plasma may reach 3.4% in early pregnancy and 6.2% in late pregnancy, however, there is a much higher percentage of maternal DNA in the plasma. Provided that the skilled artisan obtained a positive result for detection of the nucleic acid, it would require undue experimentation determine whether the nucleic acid was a results of the maternal DNA found in the maternal plasma or whether in fact the nucleic acid was from the fetus. Thus, detection of a maternally inherited nucleic acid would be unpredictable and require undue experimentation.

Thus for the reasons above and those already of record, the rejection is maintained.

Claim Rejections - 35 USC § 102

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

5. Claim 17 is rejected under 35 U.S.C. 102(a) as being anticipated by Lo (Lancet, August 1997).

It is noted that the authorship of the Lo et al. reference is distinct from the inventorship of the instant application and that this rejection may be overcome by the filing of a 132 Katz-type declaration. This rejection applies to the claims because as discussed previously this claim does not have foreign priority to the March 4, 1997 British patent application.

Lo et al. (herein referred to as Lo) teaches the detection of fetal DNA in maternal plasma and serum (abstract). Lo further teaches the detection of DYS14 from the Y chromosome (pg. 486, col. 1, para. 2)(limitations of Claim 7). Lo teaches that fetal DNA increases as gestation progresses (pg. 487, col. 1, para. 3)(limitations of Claim 17).

Response to Arguments

The response traverses the rejection. The response asserts that priority should be granted a discussed above in the priority section and thus the rejection is not applicable. This argument has been reviewed but is not convincing because the priority document does not support all of the claims. The priority document states "detection and monitoring of pregnancy-associated conditions such as pre-eclampsia which may result in differing amounts of foetal DNA being present in the maternal serum or plasma" (pg. 2, lines 24-27). This statement which proposing that variation occurs, does not provide any specific evidence that variations in fact exist, nor provides the variations from normal as stated in Claim 17. Secondly, based upon the inventive nature presumed for the invention, the skilled artisan would not have been aware that fetal DNA existed in the serum/plasma, and thus would not have "been aware that foetal DNA shows a variation over the course of pregnancy". Finally, it is acknowledged that

"a comparison" would be desirable, however, the comparison was not performed in the foreign document.

Thus for the reasons above and those already of record, the rejection is maintained.

Conclusion

6. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

7. No Claims allowable.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Enewold Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Thursday from 7:00AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jeanine Enewold Goldberg
November 1, 2000

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600
11/1/00

1601

FAX RECEIVED

DEC 27 2000

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FACSIMILE COVER SHEET OFFICIAL

TO: Examiner J. Enewold Goldberg, Group 1655 FAX NO.: 703-305-3014

FROM: C. Frederick Koenig III, Esquire; Registration No. 29,662

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	
	Lo et al.
Application No.:	09/380,696
Filed:	November 29, 1999
For:	NON-INVASIVE PRENATAL DIAGNOSIS
Group:	1655
Examiner:	J. Enewold Goldberg

Our File: JAK-PT001
Formerly SHP-PT048

Date: December 27, 2000

COMMENTS: AFTER FINAL AMENDMENT

NUMBER OF PAGES INCLUDING THIS COVER SHEET: 7

NOTIFY AMY MCSHEA IF TRANSMISSION IS NOT COMPLETE OR LEGIBLE.

I hereby certify that this paper is being facsimile transmitted to the United States Patent and Trademark Office on December 27, 2000.

Amy F. McShea

Date

Volpe and Koenig, P.C. Revision of PTO/SB/17 (11-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2001

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($) 0.00

Complete if Known	
Application Number	09/380,696
Filing Date	November 29, 1999
First Named Inventor	Lo et al.
Examiner Name	J. Enewold Goldberg
Group Art Unit	1655
Attorney Docket No.	JAK-PT001 (Formerly SHP-PT048)

METHOD OF PAYMENT

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit Account Number: 22-0493

Deposit Account Name: Volpe and Koenig, P.C.

☒ Charge Any Deficiency or Credit any Overpayment in the Total Fees Associated with this Communication

☒ Applicant claims small entity status. See 37 CFR 1.27

2. ☐ **Payment Enclosed:**
☐ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	710	201	355	Utility filing fee	
106	320	206	160	Design filing fee	
107	490	207	245	Plant filing fee	
108	710	208	355	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 0.00

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below	Fee Paid
Total Claims	28 - 28 **	0	X 9	= 0
Independent Claims	3 - 3 **	0	X 40	= 0
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	80	202	40	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	80	209	40	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	390	216	195	Extension for reply within second month	
117	890	217	445	Extension for reply within third month	
118	1,390	218	695	Extension for reply within fourth month	
128	1,890	228	945	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
142	1,240	242	620	Utility issue fee (or reissue)	
143	440	243	220	Design issue fee	
144	600	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	710	249	355	For each additional invention to be examined (37 CFR § 1.129(b))	
179	710	279	355	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	
Other fee (specify)					

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 0.00

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	C. Frederick Koenig III, Esquire	Registration No. (Attorney/Agent)	29,662	Telephone	215-568-6400
Signature				Date	

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Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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PTO/SB/21 (5-98)
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/380,696
Filing Date	November 29, 1999
First Named Inventor	Lo et al.
Group Art Unit	1655
Examiner Name	J. Enewold Goldberg
Total Number of Pages in This Submission: 6	Attorney Docket Number: JAK-PT001 (Formerly SHP-PT048)

ENCLOSURES *(check all that apply)*

- [x] Fee Transmittal Form
  - [ ] Fee Attached
- [x] Amendment / Response
  - [x] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition Routing Slip (PTO/SB/69) and Accompanying Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Small Entity Status Claimed
- [ ] Request for Refund
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [ ] Additional Enclosure(s) *(please identify below)*:

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	C. Frederick Koenig III, Esquire Reg. No. 29,662 Volpe and Koenig, P.C.
Signature	
Date	12/27/00

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box AF, Commissioner for Patents, Washington, D.C. 20231 on this date: December 27, 2000

Typed or printed name	C. Frederick Koenig III, Esquire		
Signature		Date	12/27/00

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be send to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

13/C
B. Webb 1/4/01
(DC)

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	Lo et al.
Application No.:	09/380,696
Filed:	November 29, 1999
For:	NON-INVASIVE PRENATAL DIAGNOSIS
Group:	1655
Examiner:	J. Enewold Goldberg

Our File: JAK-PT001
Formerly SHP-PT048

Date: December 27, 2000

AMENDMENT AFTER FINAL PURSUANT TO 37 C.F.R. § 1.116

Box AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

This Reply is responsive to the Action dated November 2, 2000 and the telephone conference with the Examiner on October 26, 2000. Please amend the application as follows:

IN THE SPECIFICATION

On page 1, between lines 6 and 7, please insert the heading: --BACKGROUND OF THE INVENTION--.

On page 2, between lines 4 and 5, please insert the heading: --SUMMARY AND OBJECTS OF THE INVENTION--.

OK to Enter
JEG
1/17/01
B. Webb
1/22/01

Applicant: Lo et al.
Application No.: 09/380,696

On page 6, between lines 6 and 7, please insert the heading: --BRIEF DESCRIPTION OF THE DRAWINGS--,

On page 6, line 12, please delete "Figure 3 shows" and insert therefor --Figures 3A and 3B show--.

On page 6, between lines 15 and 16, please insert the heading: --DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS--.

On page 34, line 11, please delete "Figure 3" and insert therefor --Figures 3A and 3B--.

On page 39, line 1, please delete "CLAIMS" and substitute therefor --What is claimed is:--.

IN THE CLAIMS

In claim 1, line 1, after "A", please insert --nucleic acid--.

In claim 1, line 3, after "a", please insert --paternally inherited--.

In claim 5 as amended, line 1, please delete "1" and insert therefor --2--.

In claim 25, line 4, after "for", please insert --paternally inherited--.

Please amend claim 26 as follows:

26. (Amended) A method of performing a prenatal diagnosis on a maternal blood sample, which method comprises obtaining a non-cellular fraction of the blood sample and

performing nucleic acid analysis on the fraction to detect paternally inherited fetal nucleic acid.

REMARKS

Applicants wish to thank Examiner Enewold Goldberg for the courtesy extended during the telephone interview on October 26, 2000. At that time, the Examiner advised that the claims would be allowable if limited to "paternally inherited" nucleic acid, since the specification is enabling for detecting paternally inherited nucleic acid in maternal serum or plasma. Such enablement is also indicated in the outstanding Action.

Applicants have amended the claims in accordance with the Examiner's suggestions made on October 26, 2000 and believe that the amendments to the claims place them in condition for allowance. Since these issues were previously discussed during the prosecution of this application, it is respectfully submitted that it is proper to enter the claim amendments at this time, since they eliminate issues and should place this case in condition for allowance.

While the Examiner had made specific suggestions for amending claim 1, which Applicants have adopted, the Examiner did not make specific suggestions with respect to claims 25 and 26. Applicants have attempted to amend claims 25 and 26 in the spirit of claim 1 to be in allowable form. If however, the Examiner has additional suggestions for

amendments to claims 25 and 26, Applicants respectfully request the Examiner to telephone Applicants' undersigned attorney with respect to any suggestions.

Although the outstanding Action does not reference objections to the specification, Examiner Enewold Goldberg did note that standard headings were missing and Figure 3 is illustrated in two parts, i.e. Figure 3A and 3B during the October 26, 2000 telephone discussion. Appropriate amendment to the specification has been made to address these issues. Accordingly, it is believed that this Amendment places this case in condition for allowance.

Reconsideration, entry of the Amendment and allowance are respectfully requested.

Respectfully submitted,

Lo et al.

By _____
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

Volpe and Koenig, P.C.
Suite 400, One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/amc

Interview Summary	**Application No.** 09/380,696	**Applicant(s)** LO ET AL.	
	Examiner Jeanine A Enewold Goldberg	**Art Unit** 1655	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Jeanine A Enewold Goldberg*. (3)_____.

(2) *Frederick Koenig*. (4)_____.

Date of Interview: *11 January 2001* .

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: .

Claim(s) discussed: _____ .

Identification of prior art discussed: .

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *The examiner called to discuss the after final amendment. While the applicants have provided the necessary changes, the examiner upon further consideration believes that an amplification step is a necessity for the claimed invention. The examiner indicated that prosecution would be reopened* .

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

i)☐ It is not necessary for applicant to provide a separate record of the substance of the interview(if box is checked).

Unless the paragraph above has been checked, THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

J. Goldberg
Examiner's signature, if required

15/D
B. Webb
2/9/01
(NE)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the **PATENT APPLICATION** of:	
	Lo et al.
Application No.:	09/380,696
Filed:	November 29, 1999
For:	NON-INVASIVE PRENATAL DIAGNOSIS
Group:	1655
Examiner:	Jeanine Enewold Goldberg

Our File: JAK-PT001

Date: January 24, 2001

SUPPLEMENTAL REPLY AFTER FINAL PURSUANT TO 37 C.F.R. §1.116

Box AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

A Final Action was issued November 2, 2000. This Supplemental Reply is responsive to the Examiner's telephone request of January 23, 2001 for the submission of an appropriate sequence listing per 37 C.F.R. §§1.821-1.825. Please amend the application as follows:

IN THE SPECIFICATION

Please amend the specification by entering the enclosed paper copy of a Sequence Listing (3 pages.).

On page 17, line 20, after "CAG-3'", please insert -- [SEQ ID NO: 10] --.

On page 17, line 23, please delete "10" and substitute therefor -- 11 --.

REMARKS

Applicants wish to thank the Examiner for the courtesy extended in conjunction with arriving at allowable claims and the several telephone discussions conducted during the prosecution of this application.

Pursuant to the Examiner's telephone request, submitted herewith are paper and computer-readable copies of an appropriate "Sequence Listing". The content of the paper and computer-readable copies are the same and include no new matter. An appropriate amendment has been made regarding Sequence ID Nos. 10 and 11 on page 17. No new matter has been added.

Since an agreement has been reached with respect to the allowability of all pending claims per the Examiner's fax of January 16, 2001, it is respectfully submitted that this case is now in condition for allowance. Reconsideration, entry of this amendment and allowance of the claims is respectfully requested.

Respectfully submitted,

Lo et al.

By_____
C. Frederick Koenig III, Esquire
Registration No. 29,662
(215) 568-6400

Volpe and Koenig, P.C.
Suite 400, One Penn Center
1617 John F. Kennedy Boulevard
Philadelphia, PA 19103

CFK/fap

SEQUENCE LISTING

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WAINSCOAT, JAMES STEPHEN

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